

Contrast in budget styles

India and Britain last week published taxation budgets for the year ahead. The underlying objectives are closely similar. But the Indian budget is much more imaginative.

ONE of the legacies of British rule in India is that the country's financial year begins (as in Britain) on 1 April (but there are plans to change that). So it is not simply a coincidence that the governments of both India and the United Kingdom should last week have published their plans for raising revenue during the year ahead and, in India's case, for spending it as well. (The pattern of spending in Britain has already been determined.) Curiously, the two governments have been forced by circumstances into similar strategies. Both are anxious that more should be done to make industry, and especially technically advanced industry, more prosperous. For different reasons, each government is anxious to shift the burden of raising revenue from direct taxation (of incomes and corporate profits) to indirect taxes. Each government is convinced that a well-designed budget is a means by which its domestic economy can be made productive and competitive. But this year, there can be no dispute that the government of India has made a more imaginative assault on the problems facing it than has the British government. The British government, and its Chancellor of the Exchequer, Mr Nigel Lawson, have been widely criticized for cowardliness. It seems not to have been noticed that by comparison, the government of India and its finance minister, Mr V.P. Singh, have produced a dazzling performance.

India's budget for the year ahead is the most dramatic consequence yet of Mr Rajiv Gandhi's succession as Prime Minister and of his expressed determination to make India modern. As such, his first budget should be regarded not merely as a fiscal instrument but as a strategy by means of which developing countries elsewhere might seek to drag themselves by their bootstraps into the twentieth century. For India, like many other developing countries, has nourished since independence in 1947 the notion that the development of the economy can be planned from the centre. It is not intended, even now, that this goal should be abandoned. What the new government appears, however, to have recognized is that there is a distinction between the process of channelling resources in socially desirable directions (such as the improvement of education or relief of poverty) and the attempt to control in detail the economic behaviour of the people who must be the instruments of change.

Spending

On the spending side, the Indian government plans to follow the course that has been charted in the past five years. Briefly, it will persist with the encouragement of science and technology through its technical agencies, with functions as different as the sponsorship of space research and the provision of grants for research at universities. Those similarly dependent on the British budget for research funds will be envious of the real increase planned for the coming year in India, which uniformly exceeds 10 per cent. The central government's budget will not help solve the huge problems of India's universities, most of which are the concern of state governments, but there is always a chance that the new government's good example will bring about more general enlightenment. But perhaps the most important spending decision is negative, the announcement as part of the budget package last week that government revenues will no longer be used on such a vast scale to subsidize the Indian railways. Attempts in Bengal to organize a protest boycott by railway passengers, always destined to be self-defeating, seem nevertheless to have passed

off without trouble. Economic realism seems to have a logic of its own.

The revenue side of the Indian budget is more daring. But how can it matter, for the development of a technological economy, that a government should raise the funds it plans to spend in one way rather than in another? This is where Indian and the British analysts come close to agreement, believing that the burden of taxation can be an important determinant of the way in which funds are invested in innovation. That is why both governments have come to believe that it would be economically beneficial if incomes were less heavily taxed, and if the burden were shifted to what people spend. In reality, the Indian government has a further incentive to follow such a policy, for it is generally agreed that the incidence of personal taxation (which takes away roughly half the income of a person earning roughly \$12,000 a year) is so sharp that it can only encourage the growth of the black economy, that based on the transfer of huge sums of cash and gold between private individuals and even corporations so as to avoid the tax net altogether. The Gandhi/Singh remedy is a substantial reduction of personal taxation, counterbalanced by increased revenue-raising from other sources, among which the largest single item so far is the increase of railway fares. The result may be to tempt back to legality some of those who at present manage to avoid paying taxes by simple dishonesty.

Realism

The government of India has taken several further steps in the direction of economic realism, which is nothing but a calculation that people should be allowed as far as possible to do what their inclinations suggest, by reducing (but not yet abolishing) the mechanism by which the government decides whether private or even partly public companies should be allowed to embark on some novel manufacturing enterprise. Ironically, even after the budget had been published at the weekend, last week's newspapers in India still carried statutorily required advertisements by Indian companies declaring their ambitions to branch out in new directions, and asking for objectors to declare themselves. The rationale for these regulations is straightforward; economic resources should not be diverted into activities that do not serve the general purpose. The problem on which India has repeatedly broken its ambitions in the past forty years is that civil servants are not much good at deciding what directions the development of industry should follow but are certainly slower than industrialists to make up their minds. And the system of industrial licensing has become one of the most flagrant foci for the corruption that has become endemic. By this attempt at letting people decide for themselves, the new Indian government may be following the present British government, but it may claim credit for having taken a gigantic step in one.

So what can be wrong with such a brave assault on convention? The snag is that the Indian government, following that of the United States, is planning deliberately to overspend its resources in the year ahead by about ten per cent, in round numbers the equivalent in rupees of \$3,000 million, or between one and two per cent of President Reagan's budget deficit. Even so, such modest profligacy is a gamble. The experience of recent years has shown that the states usually manage to end up overdrawn with their bank, the central government, and that budget



deficits usually double between one April and the next. This time the government must hope that economic freedom will abate this tendency. If not, there will be inflation, the poor will feel even more poor and the momentum of development will be interrupted. Even the government acknowledges that it is betting on a favourable monsoon. We should all keep our fingers crossed.

Mr Nigel Lawson will quickly plead that a British finance minister could not dream of taking such risks; for one thing, what with the developed luxury of a welfare system, his government's spending is a much larger proportion of the national wealth. Yet Mr Lawson's problem for the past year has been very much like Mr V.P. Singh's: how to create a climate in which such wealth as there may be is directed towards productive investment, preferably in innovation. The pre-budget calculation seems to have been that a replacement of direct by indirect taxation would give saving an edge, and that it would be beneficial if the favourite media for personal savings (mortgages and pensions) could be put on an equal footing with investments in risky ventures. In the event, the British government found it lacked the courage to give such offence to its electors. Nor could it go along with the entreaties of its political opponents (not to mention a substantial section of its own political party) that the annual deficit should be deliberately increased so as to stimulate demand. (Relatively credit-worthy Britain's deficit will nevertheless be twice India's.) So Mr Lawson emerges as a piteous character, "boxed in" as he describes himself. No doubt he will be laughing if India comes a cropper, perhaps because the monsoon fails in June. But will he not be furious with himself, perhaps even as furious as his taxpayers will be with him, if India's gamble comes off when he can boast only that he did not think it the right time to make a risky budget? □

New star wars row

The private dispute at Geneva over star wars may yet be drowned by rows between allies.

MR Richard Perle, Assistant Secretary of Defense at the Pentagon, is one of the US administration's hard men, and was probably hired for that attribute, not for his skill as a diplomatist. But even so, his performance in London last week inevitably raises the question whether his operating licence should be amended.

The circumstances are very odd. Two weeks ago, Sir Geoffrey Howe, the British Foreign Secretary, had made a speech in which he made quite clear his support for research under the umbrella of the Strategic Defense Initiative and then went on to muse about the problems that would arise if ever the time came to deploy an effective defence against ballistic missiles. There would have to be negotiations with the Soviet Union about the scale and manner in which this would be done. And even when agreement had been reached, there would remain difficulties about the true measure of security an apparently leak-tight umbrella could provide, if only because the present familiarity of strategic doctrines, bizarre though they may seem, would be undermined. Sir Geoffrey went on to reflect that the difficulties he foresaw might never arise because the research programme might demonstrate that the goal is unattainable. None of this conflicts with what is understood to be the objective of the research now under way, at least since the British prime minister's pre-Christmas visit to Washington last December.

So why should Mr Perle promptly launch an attack on Sir Geoffrey at a conference held in Britain just a few days afterwards? The most charitable explanation is that he read only a bowdlerized version of Sir Geoffrey's speech, or that he was himself misquoted, or quoted out of context. Yet another explanation, so mundane that it must seem an insult to such a person as Mr Perle, is that he was jet-lagged. Yet people in his trade do not usually set about supposedly friendly statesmen with such vigour, and in public, so that some kind of explanation is essential. Otherwise, as Mr Perle must by now recognize, there is a danger that listeners will think there must be more to star wars, even now, than mere research. □

Illegality proliferates

The US administration should be more diligent in preventing nuclear proliferation

FOR an administration that has been concerned — some would say obsessed — with halting the flow of US technology to the East, the Reagan Administration has been curiously apathetic about nuclear non-proliferation. So it comes as no surprise that its apathy should set the tone for the government's law-enforcement officials responsible for federal statutes designed to prevent sensitive nuclear-weapons technology from reaching non-weapons states. There is no other explanation for the strange case of a Pakistan national arrested last year when he attempted to ship home 50 high-speed switches that just happen to be of a kind needed for building an atomic bomb. Nazir Ahmed Vaid was allowed to plead guilty to one count of violating US export laws; he received a suspended sentence and was quickly deported. Although the original indictment had made clear that the switches were sensitive nuclear technology and suggested that Vaid was working on behalf of the Pakistan government, all such references were dropped in the plea bargain. The judge who approved the plea bargain said there was no evidence that Vaid was a foreign agent; he was merely a businessman "expediting what he thought was a business deal". The federal prosecutors agreed.

An investigation by the *New York Times* has now uncovered a very different story. In fact, the government had in its hands proof that Vaid was acting directly as an agent for one S.A. Butt, who happens to be the director of supply and procurement for the Pakistan Atomic Energy Commission. Telexes from Vaid to Butt make it clear that Vaid knew it was illegal to export the switches without a licence, that he was working on "alternate ways to buy these products" after a direct approach to the sole manufacturer failed and asking what he should say the "ultimate consumer" of the products was. Butt cabled back that he should say the ultimate consumer was the research and development department of Islamabad University. The prosecutors told the *Times* reporter that they had not appreciated the significance of these telexes because they had not been able to find out who Butt was. (He had no record in the US Department of Treasury crime computer, they said.) Butt is in fact well known in arms control circles as the man who successfully procured reprocessing and enrichment technology for Pakistan from Europe in the 1970s. Had these facts come out at a trial, Vaid could have been sentenced to as much as 20 years' imprisonment for violating the Atomic Energy Act.

Precisely what Pakistan is about is anybody's guess. Since the Indian explosion in May 1974, Pakistan has encouraged speculation that it is on the way to making some kind of bomb. Most probably it has been doing its best, with help from where it could be had, people like Vaid included. On present form, dependent as it is on the United States for delivery systems, Pakistan could not realistically hope to be an independent nuclear power, but it could reasonably hope to be able to make trouble. For the past five years, the United States has been ambivalent, reckoning Pakistan an ally on Afghanistan. Until recently and perhaps still, it has fudged the nuclear issue.

It is not necessary to subscribe to conspiracy theories to see a connection between the administration's laxity on nuclear non-proliferation and the laxity of a federal prosecutor in Houston in pursuing this case vigorously. Prosecutors have a limited amount of time to devote to too many cases; they are not going to knock themselves out on cases that will not win them approval at a higher level. The administration has unmistakably set the tone, a blend of laxity and of contempt for the law, or at least for the spirit of President Carter's Nuclear Non-Proliferation Act. The act may rashly have offended governments which believed that their obligations under the Non-Proliferation Treaty (NPT) were sufficient and may unwisely have overridden legal contracts, but is there any reason why it should not apply to countries which have scorned the NPT and which have no other claims on the United States? □

British research

Research council offers hope for astronomers

THE worst fears of the British astronomical community, that Britain might abandon observation in the Southern Hemisphere, may have been allayed by last week's meeting of the Science and Engineering Research Council (SERC). Some consider that the council was favourably disposed to the submission of its Astronomy, Space and Radio (ASR) Board for funds over the next five years, at a time when a combination of exchange rate fluctuations and pressure on the council's budget from other areas of science and from engineering have never been so acute. But the forward-look process has revealed differing perceptions of priority within the council's committees. Moreover, the long-term outlook is not yet certain, as the Advisory Board for the Research Councils (ABRC) has yet to consider the council's proposals, and some of the options involve international agreements.

By all accounts, the January meeting of the ASR board was an emotionally charged affair. Faced with the task of producing a list of priorities for the council, the board's subcommittees had thought the unthinkable and ordered their respective research programmes on strictly scientific grounds. It was the ASR's board's task to co-ordinate these shopping lists into a package for presentation to the council. In the event, there was a heated debate over proposals for ground-based optical astronomy.

Two of the most important telescopes currently at the disposal of British astronomers are the Anglo-Australian Telescope (AAT) and the UK Schmidt telescope, both in Australia. Both have proved highly successful and are generally agreed to be capable of producing world class results for several years yet. In the next few years, however, two new telescopes on La Palma in the Canary Islands — the 2.5m Isaac Newton and the 4.2m William Herschel — are due jointly to provide a first-class facility in the Northern Hemisphere.

At the January meeting of the ASR board, a certain polarization developed between those who favoured the retention of Southern Hemisphere activity those who felt that it would be better to concentrate resources at a variety of wavelengths in the Northern Hemisphere. This option would allow more collaboration, for example, between optical, infrared and radio astronomers, all of whom are strongly represented in Britain.

The prevailing view was that the Northern Hemisphere option would be the easier to sell to backers, chiefly on the grounds that it would be more easily

managed. The final package that went to the council was broadly split into three. The worst-case, or lower guideline option, included studentships and grants, data processing, support for approved space projects such as the X-ray satellite ROSAT and the Comet Halley explorer Giotto, the current subscription to ESA, the European Space Agency (the 5 per cent increase recently agreed will not be paid by SERC), and the Northern Hemisphere telescopes and radars. This package would represent a 10 per cent cut in current plans.

The centre guideline option, representing the current spending level, would add support for ESA's future space programme as spelt out in its "Horizon 2000" report. The

third component of the ASR board's list, and relatively low in its priorities, includes Solar System space research and Southern Hemisphere facilities.

At last week's meeting, however, the council stopped short of recommending a withdrawal from the Southern Hemisphere observatories, partly as a result of the strength of the scientific case and partly because of the international implications. Broadly speaking, the council favoured rather more than the central guideline package. One option being investigated for several of SERC's major facilities is that working time should be sold abroad to recoup costs. And, in a move that will bring weary shrugs of resignation from the staffs of the two British observatories in Sussex and at Edinburgh, yet another committee is to be set up to decide their future. This time, however, its committee is apparently to be chaired by SERC's chairman, Sir John Kingman, who is said to want this resolved before he leaves later this year.

Philip Campbell

Acid rain

US/Canada stand-off (contd)

Washington

LAST week's agreement between the United States and Canada on acid rain appears to represent no shift in the Reagan administration's policy: research, yes; controls, no. Canada, meanwhile, is accelerating its unilateral efforts to reduce emissions sources responsible for acid deposition without waiting for reciprocal action from the United States.

What last week's agreement does is to create two "special envoys" who will "examine" the acid rain issue and report within a year. The Canadians hope they will become negotiators; but the announcement suggests their role will be limited to consultation. For months, the Canadian government had made clear that acid rain would be at the top of the summit agenda. And while Canadian officials say the agreement is a hopeful sign, environmentalists have already dismissed it as a contrivance that allows Prime Minister Brian Mulroney, under considerable domestic pressure to achieve results on the acid rain problem, to claim that he did not come away completely empty-handed.

Earlier this month, however, the Canadian government was able to take credit for some more tangible steps towards acid-rain control. The federal government agreed to allocate \$150 million to aid the smelting industry, Canada's major contributor to acid deposition, to reduce sulphur dioxide emissions. The provincial governments and industry will provide the remaining portion of the estimated \$1,500-2,000 million. And, although it is only a minor source of the problem, Canada will as of September 1987 require NO_x emissions from cars and light trucks to match the stricter US standards; this is

seen more as a political move to remove US objections that Canada was demanding US action while refusing to clean up its own house.

The Canadian government says that these steps will enable the country to reach its earlier-stated goal of reducing wet sulphur deposition to 20 kg per hectare per year in the sensitive eastern provinces — but only if the United States takes "compatible" action. Canada's reduction plans, which will cut total sulphur dioxide emissions by approximately one-half, will be completed by 1994.

Although US officials do not challenge the Canadian assertion that at least 50 per cent of acid deposition in eastern Canada is the result of emissions from US power plants (while only 10 per cent of acid deposition in the northeastern United States originates in Canada), the Reagan administration insists that more research must be done before the expense of new sulphur controls can be justified. The US argument is that not enough is known about the linearity of the atmospheric response or about how quickly lakes subjected to acid deposition actually acidify. If the lakes respond very quickly, they argue, then it is possible that virtually all susceptible lakes are already acidified, and new emission controls will make no difference.

Canada has been pressing for a 50 per cent reduction in US sulphur emissions from appropriate regions. A plan proposed by former Environmental Protection Agency administrator William Ruckelshaus but rejected by the Office of Management and Budget would have cut emissions from midwestern power plants by some 3-5 million tons, which Canada says would have been sufficient. **Stephen Budiansky**

Exhaust emissions

Europe's fiercest battle yet?

Brussels

THE political agreement to clean up car pollution in Europe reached by European governments in the long night of 20/21 March may yet be regarded as one of Europe's fiercest battles. The upshot is that there will be obligatory lead-free motor fuel throughout the Community by 1 October 1989 and, the calculation goes, a 60 per cent reduction of exhaust emissions against the yardstick of 1985. The surprise is that this solution, very much a compromise, was reached without loss of face for any member government.

The need for an agreement of some kind stems from the determination of West Germany to reduce exhaust emissions quickly, and in particular from the West German threat (uttered last year) of a unilateral introduction this July of lead-free motor fuel. In the event, West Germany went along with a package of proposals that will allow different categories of cars (classified by engine capacity) to be dealt with differently. The United Kingdom seems especially proud of having won this concession and, in particular, agreement that engines of medium capacity (between 1.4 and 2 litres) may be dealt with not by catalytic converters but by the application of novel technology as represented by "lean-burn" engines (see below).

The new standards will first make their impact generally in 1988, when new models of cars with engines above 2 litres capacity will have to conform with the new emissions standards. By 1 October of the following year, all new cars sold will have to conform. In the intermediate range, new emissions standards will apply to new models from 1 October 1991 and to new cars appearing on the roads from 1 October 1993. For smaller cars, with a capacity of

less than 1.4 litres, the emissions standards are to be introduced in two stages, with a provisional standard coming into force in 1990 (for new models) and a more rigorous standard three or four years later.

Part of the uncertainty remaining about emissions standards arises because of the difficulty in defining the permissible limits of exhaust emissions from engines of different sizes. The intention, however, is that the European testing procedures will progressively be made to conform with US standards within the next few years.

One reason why last week's negotiation was successful is that West Germany eventually won the blessing of its partners for its scheme to offer financial incentives to owners of vehicles conforming with improved emissions standards. These incentives will apply from 1 July this year, but it has now been agreed that, to qualify, the exhaust emissions of small cars must be at least 15 per cent less than the transitional standards set for 1990. Moreover, the financial incentive must not exceed DM750 over a three-year period rather than the DM1,700 originally proposed by West Germany.

The underlying issue here, to which the French environment minister Huguette Bouchardeau paid most attention at last week's meeting, is that financial compensation should be "significantly less" than the cost of clean technology if it is not to contravene the Community's rules on the provision of direct subsidies for manufactured products, not to mention the environmental doctrine of "polluter pays".

The financial incentives to be offered by West Germany will include a DM0.04 per litre subsidy on lead-free motor fuel, to be introduced next week and a ten-year exemption from road-fund tax for drivers

Catalytic converters or lean-burn?

Brussels

THE British arguments against catalytic converters for smaller engines are both economic and technical. It has been estimated that a catalytic converter would cost £500 at the outset, and would involve a maintenance outlay of £90 with possible replacement after four years costing £150. Allowing for fuel penalty, the cost at Community level would be 15,000 million ECU a year if catalytic converters were the only means of conforming with emissions standards.

The United Kingdom argues that a universal requirement of catalytic converters would further open up the European market to the Japanese, whose products already conform with US standards. The investment needed for the two production lines would be a burden on ailing European car industries, while the cost of catalytic

converters would depress demand.

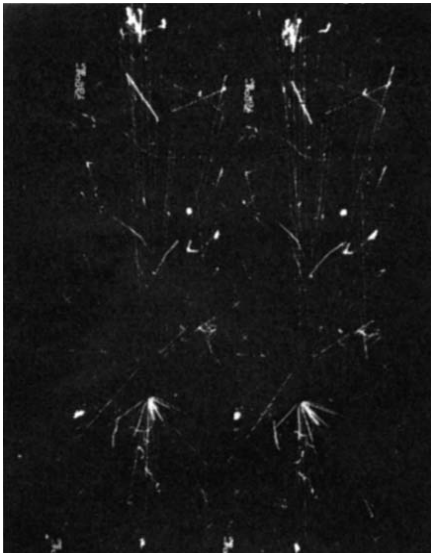
Rather than forcing the car industry down this cul de sac, the British have been arguing for time in which to develop lean-burn engines, working on the principle of altering the fuel/air ratio, thus provoking earlier combustion. The result is a fuel saving of about 15 per cent as opposed to the 5 per cent fuel penalty with catalytic converters. A prototype by Ford UK is to be demonstrated in June and could be on sale in September. The added cost to the consumer of the lean-burn engine is estimated at between £50 and £70.

The high air/fuel ratio of the lean-burn engine lowers both carbon monoxide and nitrous oxide emissions, but increases hydrocarbon emissions, for which reason an oxidation catalyst costing up to £50 would still have to be fitted.

Anna Lubinska

CERN collision

ONE of the first events taken in a streamer chamber at the European Organisation for Nuclear Research, CERN, last week, showing a proton colliding with an antiproton at 900 GeV in the centre of mass, the highest energy for such controlled collisions yet observed. Despite the complicated manoeuvres required to reach such



energies, the collider is working well, with beam lifetimes of seven hours, double what was expected, and luminosities of $3 \times 10^{26} \text{ cm}^{-2} \text{ s}^{-1}$. The plans were to introduce a gamma-ray converter around the beam pipe on Friday, to seek high multiplicity events without gamma ray production, first detected in cosmic ray observations — the Centauro events (see *Nature* 21 March, p.221).

Robert Walgate

equipped for clean driving from 1 July. Friedrich Zimmermann, West German minister for the interior, admitted after the negotiating session last week that "going it alone would have meant chaos".

The United Kingdom, at the outset one of the strongest opponents of the West German initiative, also seemed content with the outcome. For cars above 2 litres engine capacity, British manufacturers seem already to have accepted that catalytic converters will be necessary. Indeed, most car models in this range depend for their economic success on export sales, for which purpose catalytic converters may already be fitted. But the United Kingdom is also pleased with the concession that lean-burn engines may be fitted to smaller vehicles as an alternative to catalytic converters.

The Community's agreement on vehicle emissions will be promulgated by means of a directive from the Commission, due to be agreed on 30 June. For the time being, diesel-powered engines and vehicles weighing more than 3.7 tonnes are not covered by emission standards, but proposals are expected by the end of the year.

Anna Lubinska

Infant mortality

US improvement halted

Washington

A SUDDEN halt in the improvement of US infant mortality statistics after two decades of dramatic progress is turning into a political issue for the Reagan administration as critics charge that cuts in social programmes are to blame.

From 1965 to 1981, infant deaths declined from 24.7 per 1,000 live births to 11.9. But in 1982 and 1983, the rate of improvement began to level off, to 10.9 per 1,000 in 1983, the latest year for which complete statistics are available. Preliminary data suggest that there was virtually no improvement in 1984. And public health researchers say that the death rate has actually increased in about 10 states in the past few years.

After first suggesting that the slow-down was merely a statistical fluctuation, Dr Edward Brandt, just before leaving his position as Assistant Secretary for Health last December, acknowledged in a letter to state health officials that the problem was "worrisome". The administration has refused to comment directly on accusations that cuts in medical and food assistance programmes to the poor are to blame.

A study released last month by the Institute of Medicine (IOM) of the National Academy of Sciences lends credence to the critics' charges. It found a substantial link between inadequate prenatal health care and low birth-weight, the single greatest

drop that began in 1965 coincided with the start of the "War on Poverty" programmes, such as federally-funded neighbourhood health centres. And according to Dr Julius Richards, who was assistant secretary of health during the Carter administration, 725,000 people have lost services at community health centres as a result of Reagan administration budget cuts. A study in Boston found that the infant mortality rate in areas of the city served by five such centres that suffered budget cuts increased by 46 per cent in 1982 over the previous years.

The group pointed to another clue for the slowdown: while mortality rates for infants less than one month old continue to fall, the rate for infants between one month and one year old may actually be increasing. Neonatal intensive care units are allowing more low-birth-weight infants to make it through the first 30 days, but more of these high-risk infants still die within the first year of life.

The experience of other countries suggests, however, that the United States can still make considerable progress — but it will take something other than advanced medical technology and intensive care units. The United States has one of the higher infant mortality rates among the industrialized nations; much of the difference, according to the IOM study, can be explained by the difference in incidence of low birth-weights. And, perhaps, more to the point, the rate of decline in infant mortality rates in the United States (until the recent slow-down) was in line with the rate of decline in other countries with substantially lower rates, according to Dr Sam Shapiro of Johns Hopkins University, which suggests that rapid progress remains a reasonable goal.

The Public Health Service is for the time being limiting its response to assisting the states in planning studies of infant deaths and identifying possible corrective steps. With the incorporation of federal service programmes into block grants to the states under the Reagan administration, it is up to the states to take those steps.

Stephen Budiansky

European synchrotron source

More horse-trading on sites

MONEY, finally, will probably decide where and when the long-awaited European Synchrotron Radiation Source (ESRS) is built. Denmark's representatives, at the latest meeting of the "Levaux committee", established by the European Science Foundation to select the ESRS site, offered 30 per cent of the capital cost if the source went to Risø, Denmark's national nuclear laboratory. Italy's delegation offered 50 per cent if it went to Trieste, a lost corner of Italy that the government dearly wishes to develop. But the French and West Germans together proposed Grenoble, near the French Alps, and offered 68 per cent (39 per cent from France and 29 per cent from West Germany). The British could have had the casting vote (preferring Grenoble), but could offer no money. And Austria and Switzerland also professed a cashless interest in the Franco-German proposal.

Meanwhile, however, the third most important figure in the French government, socialist Louis Mermaz, president of the French national assembly, has lost his seat in Grenoble as the president of the regional assembly. It was widely supposed that it was to protect Mermaz that, at the last moment, France switched its allegiance from Strasbourg (near the German border) to Grenoble. So will the French choice now revert to Strasbourg (which Germany always preferred)? "No. I don't think so. It's decided", said a French research ministry spokesman on Monday.

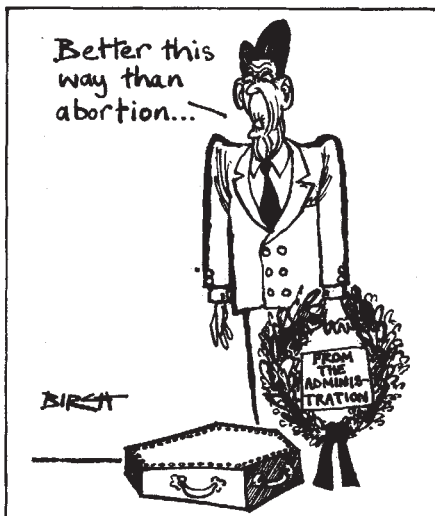
According to the French ministry, the Levaux committee heard site reports on Risø, Trieste and Grenoble which showed

them indistinguishable on all technical counts, including vibrations and geology — whereas the handful of Grenoble sites had been in question as being close to an autoroute and the Alps. "The most dangerous vibrations come from the life of the machine itself", the French ministry claims. Thus the only distinguishing factor was money, and here France and Germany are ahead.

Nevertheless, even the Grenoble proposal comes 32 per cent short of the capital required, and France and Germany will be seeking new (paying) partners. But they will make entry as easy as possible. Thus in June, France and Germany will set up a steering committee ("provisional administrative council") to which all nations are invited "even if they can't say the exact amount they will pay". Nations that admit they cannot pay will be invited as non-voting observers.

During this year, the council will nominate a director-general, set up a scientific council and seek the cash to begin construction. If the money is found, construction will begin in the summer of 1986. Could ESRS go ahead as a purely Franco-German project (as the high-flux neutron facility, the Institut Laue-Langevin, began)? Robert Comès, whom the ministry has nominated as preliminary representatives of the Centre National de la Recherche Scientifique on ESRS, says the facility "should be European", and as French political thinking is at present strongly in favour of European initiatives and research, a Franco-German breakaway seems unlikely.

Robert Walgate



risk factor in infant deaths. And the number of women visiting a physician in the first three months of pregnancy decreased in 1982 after increasing steadily during the 1970s. (The decrease was greater among black women than white women. The infant mortality rate for blacks remains twice that for whites in the United States, as it was in 1965. Black women are also about twice as likely as white women to give birth to low-weight babies.)

Those who draw a connection between the infant mortality trends and social programmes point out that the dramatic

French applied science

Researchers help but stay put

FRENCH scientists are making an effort to link up with industry — but are not prepared to quit their laboratories for a year or two to work with an industrial partner. That is the picture painted by Jean-Jacques Duby, the director for applications at the Centre National de la Recherche Scientifique (CNRS), the principal French research council for basic science, at the end of his first two years in the job.

According to Duby, his new Directorate (the Direction de la Valorisation et des Applications de la Recherche, DVAR) has helped CNRS nearly double the number of new patent applications each year by CNRS groups from 71 in 1982 to 134 in 1984, while contracts with industry have tripled in value from FF 10 million (£1 million) in 1982 to FF 31 million (£3 million) last year.

Other indicators of the application of CNRS science also show improvements, such as a doubling in consultancies (to 157). But Duby's *bête noire* is lack of mobility — with only 41 CNRS staff out of a total of over 6,000 making even a temporary move to industry in 1984, a negligible improvement on the 37 in 1982. A new contract of employment for CNRS researchers, which came into effect last year, was intended to increase mobility, by giving staff civil servant status (thus guaranteeing employment and pension rights whatever moves a person might make), but clearly it has had little effect so far.

Moves in the other direction, out of industry and into research, are also rare. Duby launched a new scheme last year to grant "associate directorships of research" to industrialists wishing to spend time in CNRS laboratories. Just six such positions are about to be announced. The French, whose economy is still more agricultural and land-based than most in Europe, like to stick to their territory.

Nevertheless, there has been at least a psychological change in the relationship between French scientists and industry. The year before Duby came to CNRS, a survey of decision-makers showed that the CNRS profile had fallen "very low". But "now we even get phone calls from the big companies", says Duby. This is a "drastic improvement" on the past. But the smaller companies are still largely unaware of CNRS, and even the big companies are very wary "at risk point", when they must decide whether to invest money with CNRS in a project. "We lack venture or any other capital in France", says Duby. Another problem is that French law centres on institutions rather than contracts, so new ventures tend to require new institutions.

Despite these obstacles, Duby's DVAR has been trying every possible method of making a profit out of CNRS science, often with success. Some FF 2.8 million (£280,000) was redistributed to 54 CNRS laboratories last year from profits from

patents alone. And the organization has created companies — under its new constitution, it is free to invest and make profits — such as MIDIROBOTS, a robotics company in the south of France.

Interest from industry is as great in fundamental physics as in the engineering sciences, Duby says. Organic chemistry and pharmaceuticals (where the companies have money to spend) lead the field, followed by materials science. Interest in electronics, data processing and mathematics has been "disappointing", although Duby had expected demand for work on systems and modelling.

CNRS is also putting a lot of effort into biotechnology, although this faces two major problems: on the research side, groups are often controlled by an old guard of fundamentalists, little interested in application; while on the industrial side, the big companies such as Rhône-Poulenc have rigid, seven-year research programmes into which, paradoxically, it is difficult to fit new ideas.

Duby and his DVAR are also working hard with:

- LABINFO, a database on French research and researchers, now covering 5,500 laboratories in CNRS, universities and other research councils, through which industry can trace who is working on what.
- The CNRS industrial relations committee (CRIN), whose twenty working

groups discuss industrial problems and research solutions, and make proposals for research to CNRS.

- Framework contracts with companies, which cover matters such as potential patents rights, exchange of staff and payments, forming an "umbrella" under which CNRS laboratories can more rapidly reach agreement with the company on a detailed project. These contracts are being increasingly "regionalized", being signed and managed by local branches of CNRS management.

- Publicity: CNRS has been holding "industrial days" in the regions, such as one on lasers in Grenoble and one on biotechnology in Paris, and has even established an information centre at Orly airport south of Paris.

- Co-financing of applied research with the national association for the application of research, ANVAR, which works mostly with small and medium industry: this brought CNRS laboratories an extra FF 25 million (£2.5 million) in 1984.

But Duby, ever energetic, says CNRS has much more to do. The figures for CNRS/industry cooperation are simultaneously "optimistic and derisory". They are optimistic in that they are growing; derisory in that they remain too small.

Two thirds of CNRS laboratories still have no link at all with industry, and those links are with only 1,000 out of 50,000 French businesses. ANVAR, by contrast, has 5,000 such links. It is a target Duby will be aiming for.

Robert Walgate

UK higher education

Modest extra for high-tech

AN injection of £43 million into selected British universities over the next three years was announced last week by Sir Keith Joseph, Secretary of State for Education and Science (see *Nature* 7 March, p.3). The intention is that the extra money will help to bridge the expected shortfall of graduates available to the high-technology and engineering industries, particularly those involved in information technology, electronics and software. The sum to be disposed by the University Grants Committee (UGC) will be £11 million in 1985-86 and £16 million in each of the next two years.

This injection may be described as a "third time lucky" attempt by Sir Keith to fulfil an election promise to increase spending on such training. Originally, it was intended that funds would be switched within the budget of the Department of Education and Science (DES) from the arts and humanities to science and engineering. It was realized, however, that this would penalize the arts disproportionately, given the difference in unit costs between a science and an arts graduate. A second attempt to find the money was partially successful but ended in the debacle last autumn when Sir Keith was forced by a

backbench revolt embarrassedly to retract a proposal that better-off parents should pay towards the tuition fees of their offspring. This diminished the extra funds for universities from £48 million to £38 million over three years.

The third attempt was supported by the Prime Minister, Mrs Margaret Thatcher, whose involvement was made necessary because the scheme requires contributions from various government departments. Those who forked out were the Welsh and Scottish Offices, and the Departments of Energy and of Trade and Industry.

UGC is already considering bids from universities for 1985-86 funds. In subsequent years, polytechnics will also be able to compete, which would represent further encouragement after the announcement earlier this year of an allocation of £2.5 million for 1985-86 for science and technology research. Mrs Thatcher has now written to some of the industrialists whose concern over future skilled manpower shortages helped to stimulate the new funds to ask for their views on the allocation of the money available and on the prospects for more direct support for university research.

Philip Campbell

Soviet academy

Winds of change discerned

THE general meeting of the Soviet Academy of Sciences, which opened only three days after the accession of Mr Mikhail Gorbachev, was quick to respond to the tone of the new regime. In his opening speech, the president of the academy, Anatolii P. Aleksandrov, put special emphasis on Mr Gorbachev's remarks at the Central Committee plenum urging all Soviet scientists to "more active involvement in the solution of important economic, public health and cultural tasks".

Such exhortations are not, of course, new. For the past two decades, every party directive on science has urged closer ties between research and production. This time, however, there was a note of urgency not always apparent at academy meetings. In a rare moment of self-criticism (relayed, incidentally, by Moscow radio), Aleksandrov noted that since the 26th Party Congress in 1981, "many scientific developments which seemed to be economically productive, important and accessible to our industry, were made use of only after a long delay".

The lag in implementing research results in Soviet production is a well-established fact of Soviet life and seems to be endemic in the central planning system, which makes no provision for the non-productive weeks or months necessary for modernizing plants or installing new equipment. The traditional scapegoat, however, is the planner or the factory manager; for scientists to accept at least part of the responsibility is, to say the least, unusual.

Whatever the explanations, Aleksandrov went on to say, Soviet scientists must now apply their efforts to finalizing the plans for the coming five years. Although the "basic directions" have already been decided by the academy and the State Committee for Science and Technology, "certain revisions are required".

What these "revisions" will entail is not too difficult to guess. This year, Konstantin Frolov, a specialist in mechanical engineering, has been elected as an additional vice-president. Each academy vice-president is responsible for a major field of research within the academy institutes; Petr Fedoseev for social sciences, Yurii Ovchinnikov for chemical and biological sciences, Evgenii Velikhov for physical and mathematical sciences, Aleksandr Yanshin for Earth sciences and so on. Although all these sectors have their applied and technological aspects, the election of Frolov to the vice-presidency suggests a policy change that would restore to the academy the technology institutes of which it was shorn in the reforms of the early 1960s.

Another field not often stressed at academy meetings is that of military research but, at a special session devoted to the 40th anniversary of the end of the

Second World War in Europe, the contribution of Soviet scientists to the allied war effort was much lauded. Then, Aleksandrov continued, the level of development of Soviet science still directly influences the country's defence potential, countering "the military superiority of the forces of imperialism". The academy went on to adopt a resolution calling on "scientists of the world" to ensure that atomic research is directed towards peaceful purposes only and that space technology is used not for "star wars but to support the cause of peace and progress".

For the most part, however, the academy meeting ran true to form. Reports were presented, foreign contacts noted (28,500 Soviet scientists posted to socialist countries during the past five years) and the "complexities" of relationships with the United States over the past five years deplored. A few major innovations were mentioned almost in passing, in particular the establish-

ment of a centre for public opinion research at the Institute of Sociological Research. Some difficulties were glossed over. Academic secretary Georgii Skryabin correctly reported the "successful research" into computer software but said nothing of the difficulties in training computer engineers recently noted in *Izvestiya* by Vyacheslav Elutin, the All-Union Minister of Higher and Secondary Specialized Education.

Perhaps, however, the most significant tribute to the new Gorbachev era came in the TASS announcement that, as expected, Aleksandrov had been re-elected president of the academy. Leading Soviet scientists are, in general, as remote from the public as are political leaders and, at 82 and in failing health, Aleksandrov has been more remote than most. Almost ten years after his first election to the post, TASS has nevertheless decided to give him something more of a public image, Aleksandrov's career was "a flurry of brilliant ideas and decisions". "He is cheerful and sociable", TASS went on, "likes a good joke and a hearty laugh. But he is furious when he sees injustice".

Vera Rich

Soviet geneticist

Smuggling charge dropped

DR David Goldfarb, the Moscow Jewish molecular geneticist, who last year was accused of trying to smuggle Soviet "national security material" — bacteriological strains — to the West, has been informed by the KGB that the smuggling charge against him has been dropped. He is now threatened, however, with prosecution for advocating a Western boycott on his behalf.

Dr Goldfarb, whose emigration visa for Israel was apparently obtained by supporters in the Academy of Sciences bypassing the normal emigration and security channels, had that visa rescinded last April, a few days before he was due to leave. His research notes, personal papers and strains were confiscated and he was informed that the strains were the property of the Soviet Union. In fact, all the strains involved had originally been obtained from laboratories in the United Kingdom or the United States, either directly or through the good offices of Wladyslaw Kunicki-Goldfinger, or of the Polish Academy of Sciences.

Recently a group of biologists from the United States and Europe proposed a moratorium on the sending of strains to the Soviet Union until Dr Goldfarb's materials were returned to him and he was issued a new exit visa. During his recent conversation with KGB officer Gusev, Dr Goldfarb was confronted with a copy of a letter he had written to Professor Elie Wollman of the Institut Pasteur in Paris, supporting the idea of the moratorium. Gusev said that the letter might constitute a case of "anti-Soviet propaganda" and made Dr Goldfarb sign a document to that effect. The document, together with the

letter, will be forwarded to the prosecutor's office, in order to determine whether any further action is necessary. When Dr Goldfarb asked how Gusev had got hold of the letter, he was told that "someone picked it up in the street". Gusev added that, in any case, the moratorium would not be effective, since the Academy of Sciences said that only a few letters of protest had been received from the West".

Gusev then returned to Dr Goldfarb all the confiscated written materials (but not the strains), and said that the smuggling charges had been dropped. According to a representative of the New York-based Helsinki Watch Committee, this is the first known incident of the KGB returning confiscated material, or informing a suspect that the charges against him had been dropped.

In a telephone conversation with his son in New York, Dr Goldfarb said that so far it is unclear whether the return of the material indicates that he will be allowed to emigrate, or whether it merely marks a change in tactics by the KGB in response to Western reaction to the "strains affair". The Goldfarb case was raised during the negotiations last January about the possible resumption of the bilateral exchange agreement between the Soviet Academy of Science and the US National Academy of Sciences. According to Professor Alex Rich, a member of the United States team, Dr Yurii Ovchinnikov, a vice-president of the Soviet Academy said: "Of course this strain thing is not serious and we are trying to clear it up. But there is not much that we can do as it is in the hands of the KGB".

Vera Rich

UK-Japan exchange

Go East young man

Tokyo

GIVEN the near-impossibility of finding any kind of academic post, most young UK scientists would find it hard to believe there is a source of extraordinarily generous post-doctoral fellowships that never attracts enough applicants. But, according to new figures from the fellowship scheme operated by the Japanese Society for the Promotion of Science and the Royal Society of London, this is exactly the case.

The fellowships are available to support study in Japan's universities, many of which now boast enviable facilities, and would enable a foreigner to live in comparative luxury. A generous tax-free monthly stipend of nearly £1,000 (¥270,000) is provided, along with a monthly housing allowance of up to £350 (¥100,000) which would cover most of the rent of a reasonably spacious apartment in Tokyo, and to add icing on the cake there is an annual allowance of a further £350 (¥100,000). All of this is topped off with a round-trip airfare from Britain to Japan. But despite these incentives, only 32 of the 60 awards, which are for one or two years, have been taken up in the first six years of operation of the scheme: scarcely 50 per cent of the posts have been filled.

So what has gone wrong? Britain's figures make a poor showing when compared with identical schemes operated in West Germany and France. In these countries, all but one of the available fellowships have been taken up, and in 1983, for example, there were 35 qualified applicants for the eight fellowships open to French scientists.

Royal Society officials say the lack of applicants is due to the extremely tight situation for academic posts in Britain — few young scientists are willing to risk leaving Britain for the Far East for one or two years; they prefer to be on the spot for any "new blood" openings that might come up. But the employment situation is surely no better in West Germany and France.

Part of the problem may lie in fear of the unknown — language barriers and apprehension about the state of scientific research in Japan may deter potential applicants. It is true that any foreign scientist coming to Japan must accept that he or she does so as a "guest". On the plus side this means that a visiting scientist need not follow the gruelling work schedule of his Japanese counterparts, although some attempt to conform is expected — young Japanese scientists typically work 12 hours a day six or seven days a week. On the negative side, permanent posts in Japanese universities are virtually closed to foreigners; in 1982, government regulations were changed to allow the appointment of foreign nationals to university staffs in spite of their status as civil servants, but only two

foreigners have so far been appointed as permanent members of staff in Japanese national universities. But British scientists who have ventured to come to Japan under the fellowship scheme have not regretted the move, according to John Richards, science officer of the British Council in Tokyo.

Maybe the real problem is that information about the scheme has not been widely enough disseminated in Britain. In France

and West Germany, several organizations are involved in the selection procedure. In France, for example, many scientific organizations (such as CNRS and INSERM) and two ministries have representatives on the selection committee, and perhaps this more broadly based involvement of scientific and government bodies helps to draw in more applicants.

Those who criticize Japan for putting up barriers should recognize that here is a situation in which Japan has opened its doors to foreigners but few are taking the opportunity to walk in.

David Swinbanks

Asbestos

Claims centre in sight

Boston

IN the face of mounting legal costs and jammed court dockets, a group of asbestos manufacturers and insurance companies is setting up an out-of-court processing centre to handle the claims of the thousands of workers who claim to have been injured by exposure to asbestos.

After two years of negotiations, the Asbestos Claims Facility, to be based in Boston with a branch in San Francisco, will be born officially on 29 May. So far, 33 manufacturers — including the Manville Corporation, which filed bankruptcy three years ago in anticipation of financially-crippling liability judgements — and 22 insurance companies have agreed to participate.

More than 23,000 cases are now pending in state and federal courts, with about 500 new suits filed each month. A Rand Corporation study estimates that manufacturers and their insurers have already spent \$1,000 million to settle asbestos-injury claims over the past ten years, with the companies' and plaintiffs' legal expenses consuming 61 per cent of that total; legal expenses were threatening to escalate even further as the manufacturers and insurers began suing each other to determine who was responsible for paying off the successful plaintiffs. In joining the Asbestos Claims Facility, the companies have agreed to drop those battles. The insurance companies will provide the first several hundred million dollars (the exact amount is not yet certain) to pay off claims; if the insurance funds are exhausted, the manufacturers will shoulder the remaining financial burden.

Workers who choose to bring their claims to the facility will have to submit certain standard information, including employment and medical records. They will have to establish that they have suffered asbestos-related injury, such as asbestosis, and that they were exposed to asbestos-containing products made by at least one of the participating manufacturers. Facility staff will then try to negotiate a settlement. Failing that, the next step will be an offer to enter into mediation — binding or

nonbinding — with a neutral third party. If an agreement still cannot be reached, the worker will be free to take the case to court. The facility will provide legal defence on behalf of all the companies in those cases.

Claimants, who were consulted by the companies in planning the facility, stand to gain by having their cases heard much more quickly than is now the case and by lower legal costs.

Thomas Henderson, a Pittsburgh lawyer whose firm represents 500 plaintiffs, says he is cautiously optimistic.

The Reagan Administration, which has resisted calls for government intervention in asbestos cases, has been more lavish with its praise. The establishment of the facility, says Office of Management and Budget general counsel Michael Horowitz, "demonstrates the ability of the private sector to resolve serious national problems without reliance on the government to dictate and enforce its own agenda".

Tom Burroughs

Universities clouded

THE most gloomy statement yet of the prospects for British universities was uttered last Friday by the person best qualified to judge, the chairman of the University Grants Committee (UGC), Sir Peter Swinnerton-Dyer. In a statement to a joint meeting of UGC with the Committee of Vice-Chancellors and Principals, he said that the failure of Sir Keith Joseph to refer to UGC's advice on higher education last November in a recent open letter "must be a bad sign" and a proof that the advice had been rejected.

In money terms, Swinnerton-Dyer said, the British government was expecting the universities to accommodate budget erosion of 0.5 per cent a year while asking them "to do new things". But, he guessed, the new system of cash limits would amount to a cut of 2 per cent a year. At this rate, he said, it would be necessary either that university departments or that whole universities should be closed. □

Whales

UN scientist takes a hand

WHALES and other marine mammals have found a new (objective) champion — the Yugoslav marine scientist Stjepan Keckes, for long director of the United Nations' "regional seas" programme. That programme, one of the most effective of the United Nations Environment Programme (UNEP), is now to extend its work from environmental protection to a study of fisheries and marine mammals.

Thus earlier this month, Keckes convened a meeting in Geneva of all agencies supporting marine mammal research, and had armed himself with a list of 36 research projects already mounted at a cost of \$6 million and of 30 new projects, potential claimants on UNEP, which has \$390,000 to spend on marine mammals this year. The environmentalist group Greenpeace proposed a project to clean up an island fouled by nets where the rare Hawaiian monk seal breeds, but most proposals were for research.

Research is sorely needed, according to British fisheries scientist Sydney Holt, who represents the Indian Ocean islands of the Seychelles on the scientific committee of the International Whaling Commission (IWC). IWC, which is a group of whaling nations and of others opposed to whaling, has proposed a ban on whaling from next year. Holt supports the ban, but says that IWC whale population models, on which catch quotas are based, are "not worth the computer tape they are stored on". In the UNEP regional seas magazine, *Siren*, he says that the survival rate of juvenile whales is a key factor in calculations but that it cannot yet be measured. Thus the models simply assume a figure, which remains constant as a function of population and population density. "In other words, we ignore it ... all our calculations are based on models in which we ignore changes in nearly every important factor."

Holt claims, for example, that there is no certainty that right whales are increasing in the Southern Ocean, as has been claimed. And humpbacks seemed to be increasing rapidly in the North-West Atlantic only because a lot of them were seen on the coast and in fishermen's nets. "But the whales were coming inshore for food because the offshore fisheries were depleted."

Keckes believes his research programme will breathe life into the floundering "global action plan" for marine mammals which has been planned over seven years by UNEP and the Food and Agriculture Organization. One objective is to help coordinate the work of bodies such as the World Wildlife Fund and the International Union for the Conservation of Nature. Governments are to meet on the issue in Nairobi next month. **Robert Walgate**

European telecommunications

Commission plans ahead

Brussels

THE European Commission has announced plans for the preparatory phase of a major research and development programme to modernize European telecommunications. Known as Research in Advanced Communications in Europe (RACE), the programme will be considered by Community research ministers at their meeting on 4 June.

Over the years, research in telecommunications has been a central part of the Community's industrial strategy. In the late 1960s, for example, a commission under Dr Pierre Aigrain singled out telecommunications as a fruitful field for collaboration but was unable to devise a workable programme. More recently, however, the Commission has been able to develop common specifications for equipment, plans for the coordination of licensing procedures and, last October, partial liberalization of European markets for equipment (see *Nature* 311, 697; 1984).

The new programme has been put together by the Commission with the advice of national experts drawn from industry and academic life. The first objective of the preparatory phase will be to pave the way for the infrastructure needed both to support existing services and to develop new services for voice, data and video transmission. The Commission intends that there should be close collaboration between the RACE project and the Community's advanced information technology research programme, known as Esprit.

Between 1986 and 1991, work under the new programme will concentrate on integrated broad-band communications systems, when there will be some precompetitive development of equipment and services. The Commission also intends continuing the development of international standards in telecommunications.

If the first phase of the programme is agreed, the Commission would push on with a second phase between 1991 and 1996 and would then expect to invest at least 150,000 million European Currency Units (ECU) in advanced telecommunications equipment including colour telecopiers, newspaper transmission, voice-driven telecopiers and mobile videophones.

Large though this estimate may be, the Commission points out that it is small compared with the size of the telecommunications market, which is expected to amount to \$500 million a year by the turn of the century. Europe is estimated to invest 20 per cent of total expenditure on telecommunications equipment, but the Commission hopes that its programme will help Europe to break into foreign markets on a substantial scale by the next century.

Meanwhile, the Commission is planning to hold a second demonstration of video-conferencing techniques in Europe in June

this year and hopes that by May it will have developed a single standard for second-generation mobile telephones to replace the three standards now in use.

Anna Lubinska

European biotechnology

Brussels

THE governments of the European Community have now given the formal go-ahead for a four-year programme of research and training in biotechnology. As in other fields, the Community hopes to encourage transnational cooperation between academics and industrial interests in the ten member states.

Belatedly, 55 million European Currency Units (ECU) has been allocated for the period between 1985 and 1989, of which 9.5 million ECU are specifically earmarked for research in the collection of information, the modernization of databanks and the provision of access to existing facilities. As part of this effort, the Community hopes to improve existing collections of biological material and to develop better methods of storage and recovery.

Although the scale of support for the four-year programme falls one-third short of the sum originally sought by the Commission, the new programme is broader in scope than the first proposals, which concentrated on biomolecular engineering. While research in genetic engineering will still be supported, the new programme will also involve research on protein design as in the construction of artificial enzymes and the potential industrial applications of gene transfer. Applied research will include the development of reactors for converting waste lignocellulose into ethanol, from which the research directorate of the Commission has hitherto fought shy because of the uncompetitive prices of raw materials in Europe.

The underlying objective of the programme, according to the Commission, is to create a series of interlocking biotechnology research centres across Europe. But the cut in the research budget means that fewer contracts can be awarded, while it has been necessary to drop the study of the genetics of higher plants and the application of cell biology to the prevention of human disease, although work on the development of vaccines will continue.

Although the application of biotechnology in public health is an important aspect of the new programme, the chief focus of the research to be supported will be the use of agricultural products for industrial purposes, not for eating. The hope here is to find a solution to the problem of food surpluses in the Community.

Anna Lubinska

Better safe than sorry

SIR — I accept that if Enoch Powell's Unborn Children (Protection) Bill, for which I voted, becomes law it will obstruct and divert the course of research on the physiology of fertilization and implantation. But neither the Warnock committee, nor the Royal Society evidence to it, nor the Medical Research Council comments on its report, seemed to me to take sufficient account of the pace of current research in mammalian embryology and molecular genetics. Here most of the work is done on the mouse and other animals, with much of the most important fundamental work on the control of the structure of the embryo being done on the fruit fly. Many processes of development are similar across a wide range of species.

It seems unlikely that the fruits of such research will be a sharp distinction between a well-defined range of severe genetically based abnormalities such as Down's syndrome and a "normal" genetic make-up. There seems more likely to be a continuous distribution of manifold genetic variations with structural and functional consequences with varying social acceptability.

Shall we then reject an embryo which seems likely, say, to have a tendency towards schizophrenia, without our being able to tell whether there may be a genetic association with some less detectable tendency such as intelligence or aesthetic sensitivity? Would we wish to allow individuals, or the Secretary of State, or a medical panel such choices? Yet no sooner do we have the technique of *in vitro* fertilization available than the most pressing arguments are being put forward for developments in clinical research and practice to help with many clinical and humanitarian problems by refinements in the technique and its mode of use. Within the next few years, which will be the life of the Powell bill, the problems apparently susceptible to such treatment may well extend over wide areas of human formation and behavioural tendencies, affecting most people and most of life.

Is it not wise to explore much more thoroughly the mechanisms of genetic selection and manipulation before rushing in to apply them prematurely in ill-understood ways to human embryos? Is it not wise then to allow society time to think through the legal, social, moral and religious questions raised? That is certainly the overwhelming view of the lay public to whom we are answerable in the House of Commons. Those who want a greater freedom to experiment, for entirely understandable reasons, are playing directly into the hands of forces hostile to science.

There is a danger of building up a public backlash against science as a whole that will be more damaging, even within embryology and developmental biology, than any such restrictions as those of the Powell

bill. Within a fortnight of the vote on the bill, at the annual lunch of the Parliamentary and Scientific Society, Sir Keith Joseph, Secretary of State for Education and Science, was using it to take the heat off criticisms of his slaughtering of British science, sentimentally lecturing the assembled scientists about their need to take moral considerations into account in their research. It is not morality that the scientists lack, but political guile.

JEREMY BRAY

House of Commons,
London SW1A 0AA, UK

Yellow rain

SIR — In their detailed letter on the yellow rain controversy, Rosen *et al.*¹ confound their own case for chemical warfare in South-East Asia. Contending that "people indigenous to the area collected and turned in anything that was yellow, in spite of the fact that yellow rain attacks were rare by mid-1982", the authors discredit the principal source of eyewitness testimony and of environmental samples, including those in which they have reported the presence of tricothecene mycotoxins. One must be suspicious of the assertion that Hmong refugees once provided authentic samples and testimony from chemical warfare attacks and at some arbitrary time ceased to do so. If we hold Rosen *et al.* to their statement, native witnesses might well have dissembled in their accounts of chemical warfare and turned in unauthentic samples at any point from 1978 to the present.

The Hmong interviews on yellow rain, which Rosen *et al.* have obviously not consulted, offer numerous accounts of chemical attacks both before and after mid-1982. These interviews, conducted from 1979 to 1983 by a variety of agencies (refs 2-4 and R. Haruff, personal communication), show a persistent pattern: the warfare scenarios, including the repercussions of illness and death, vary considerably in content and scope, while the purported chemical warfare agent seen on the ground is invariably described as yellow and matches bee faeces⁵. While the interviews by no means offer unequivocal proof of chemical warfare, they do represent the combat experience of Hmong guerrillas in Laos.

Further, the interviews suggest Hmong refugee accommodation to their US patrons' interest in building a case against Soviet influence in South-East Asia. As the bee faeces theory implies, the transformation of Laotian attacks on the Hmong into the metaphor of yellow rain may have occurred early in the post-Vietnam War phase of US-Hmong relations⁵, leading refugees to procure descriptively appropriate samples whose aetiology was as much a mystery to them as

to Western investigators.

The complex political vulnerability of the Hmong, both in their flight from Laos and their dependence on US officials, is an integral part of the yellow rain story. If the controversy is ever to be resolved, it will require a more studious appreciation of Hmong perspective than evidenced by Rosen *et al.* in their facile repudiation of native testimony.

JEANNE GUILLEMIN

Department of Sociology,
Boston College,
Chestnut Hill, Massachusetts 02167,
USA

1. Rosen, J.D., Cohen, H., Mirocha, C.J. & Shiefer, H.B. *Nature* 313, 271-272 (1985).
2. *Use of Chemical Weapons in Southeast Asia since the Vietnam War*, Hearings before the US House of Representatives Subcommittee on Asian and Pacific Affairs, December 1979.
3. *United Nations General Assembly Report A-37-2959*, 1 December 1982.
4. *An Epidemiological Investigation of Alleged CW/BW Incidents in Southeast Asia* (Surgeon General Branch, National Defense Headquarters, Ottawa, 11 August 1982).
5. Guillemin, J. *Hmong Interviews on Yellow Rain: Cultural Rules and Political Agendas in Refugee Reports* presented at the American Anthropological Association Meeting, Denver, Colorado, 21 November 1984.

Australian databank

SIR — A computer-based system (MBIS, the Molecular Biological Information Service) for use by molecular biologists within Australia, has been put on-line by the Division of Molecular Biology of the Commonwealth Scientific, Industrial and Research Organization (CSIRO). The system can be used free of charge by authorized individuals who are either directly linked to CSIRONET (CSIRO's computing network) or through a dial-up service. Dial-up Access is charged as a local call and transmission speed can be either 300 or 1,200 bytes per second.

The system is menu driven, allowing simple access to the services available. At present, nucleotide sequence databases from GenBank, the European Molecular Biology Organization and National Biomedical Research Foundation (NBRF) are on-line as well as the NBRF protein database and are updated as soon as new versions are made available.

Bibliographic information (GenBank) is also on-line. The software library can be used to retrieve, compare and/or analyse sequence data. The uploading or downloading of information is easily accomplished. Apart from programmes written at the division, software has been made available by R. Staden and M. Kanehisa.

An electronic Notice Board and separate electronic Mail System are available which we hope will aid better communications among molecular biologists in Australia.

A.H. REISNER

CAROLYN BUCHOLTZ

MBIS
CSIRO, Division of Molecular Biology,
PO Box 184,
North Ryde, NSW 2113,
Australia

A new type of forest decline in Germany

from L. W. Blank

There is much concern about the state of West Germany's large forests which are threatened by an unprecedented decline. Research into its causes no longer focuses on acid rain, but on a possible interaction of increased ozone concentrations, acid mist and climatic factors.

IN the past few years a severe and rapidly increasing decline of forests has been observed in the Federal Republic of Germany (FRG). It is now estimated that half of the total forest area is damaged to a varying extent¹. This 'new type' forest decline, as it is called, is unprecedented in severity and geographical extent. Unlike most previous localized dieback events it cannot be attributed to a known cause.

Forest decline is now widely considered to be the most important environmental problem in the FRG, attracting considerable scientific as well as public attention. Here I present a brief account of the history of the decline, its present extent and severity, and the discussion of its potential causes.

History

First reports of damage in the early 1970s did not attract much attention or cause any serious alarm. Damage was then confined to a single species (silver fir; *Abies alba*), which accounts for only 2% of the total forest area (Table 1). Furthermore, silver fir decline is not an uncommon phenomenon in Central Europe, where localized dieback events have been well documented during the last two centuries^{2,3}. However, the recent fir decline occurred almost simultaneously at various locations in the south of the country including the Black Forest and the Bavarian Forest⁴⁻⁶ (Fig. 1). Similar fir damage has also been observed in Italy, Switzerland, Poland and in Czechoslovakia⁷⁻¹⁰.

Silver fir decline in the FRG increased dramatically during the hot and dry summer of 1976 and is now affecting nearly all stands. Main damage symptoms are: gradual discolouration and subsequent loss of needles, from the base to the top and from inner to outer branches; premature growth reduction and formation of a 'stork's nest' crown; branch dieback in the lower crown and formation of epicormic shoots; extension of normal wet heartwood into the sapwood at the base of the tree ('wet core'); damage to the fine root system.

Since the late 1970s damage has also been noticed on other tree species. Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*), and common beech (*Fagus*

sylvatica), which together account for 77% of the total forest area (Table 1), have started to show signs of damage. Symptoms are similar but not identical to those reported for fir; a comprehensive list of symptoms is given by VDI⁷. Damage to Norway spruce, the dominant species in German forests where it forms 40% of forest plantations, is of particular importance, not the least because of its considerable economic value. Monitoring plots in severely affected areas of Baden-Württemberg showed a rapid increase in damage to spruce from 6% in 1981 to 94% in 1983¹¹. Similar plots do not exist for other parts of the country, and reliable data on the increase of damage are thus rare.

Damage was initially restricted to higher altitudes (above 500 metres), affecting older

trees more severely than younger ones. Although there still appear to be patterns of damage due to altitude and age the variability between individual trees is so great that severely affected trees can be found next to apparently healthy ones at most sites.

Present severity

To obtain a clear picture of the extent and severity of damage the Federal Minister of Food, Agriculture and Forestry initiated forest damage inventories which were carried out by the individual federal states in the summer of 1982, 1983 and 1984. Forests were assessed using a set of criteria detailing damage symptoms for all relevant species¹². This verbal description has since 1983 been complemented by a set of colour

Fig. 1 Major forest areas (triangles) and urban and industrial conurbations (hatched areas) in the Federal Republic of Germany.



photographs¹³. Extent and severity of damage were classified using a system of damage categories. The initial system of three categories (category I, slightly damaged; category II, damaged; category III, severely damaged) as used in 1982 and 1983 was extended in 1984 by adding a category 0: 'without damage symptoms' and category 4: 'dead'. The main criterion for damage classification was the percentage of needle loss, which was related to damage categories. Trees with a loss of up to 10% were considered unaffected (category 0), 10–25% loss indicated slight damage (category I), 26–60% medium damage (category 2), and more than 60% loss severe damage (category 3). A second important criterion for damage classification was the discolouration of the foliage.

Before comparing the results of the three successive inventories, it is important to note that there are marked differences in their methodology and consequently in the accuracy of their damage assessment. The first survey in 1982 was largely an estimate, covering only parts of the country. The following survey in 1983, which covered the whole of the Federal Republic, used more detailed assessment criteria, and three

Table 1 Area and species composition of forests in the FRG

Species	Area (ha × 10 ⁶)	Proportion of total forest area (%)
Spruce	2.951	40
Pine	1.464	20
Fir	0.176	2
Beech	1.250	17
Oak	0.615	8
Others	0.950	13
Total	7.406	100

The total area of the FRG is 244,000 km²; approximately 30% of this area is covered by forests.

federal states based their survey on spot-checks at assessment areas. This system was adopted on a national basis for the 1984 inventory, with a 4 by 4 km grid system determining test areas. The results obtained in these areas were extrapolated and presented as percentage damage to each tree species, to certain forest areas or to the total forest area.

Perhaps the most striking impression gained from a comparison of the three inventories is the drastic increase in the total damage forest area from 8% in 1982 to 34% in 1983 and 50% in 1984 (Table 2)^{1,14}. This is mainly due to an increase in damage category I (slight damage) from 6% to 25% and now 33%, and an increase in category II (damaged) from 1.5% to 9%, and now 16%. The percentage of severely damaged and dead trees has risen only slightly from 0.5% in 1982 to 1.5% in 1984. Coniferous trees are generally more affected than deciduous species, but

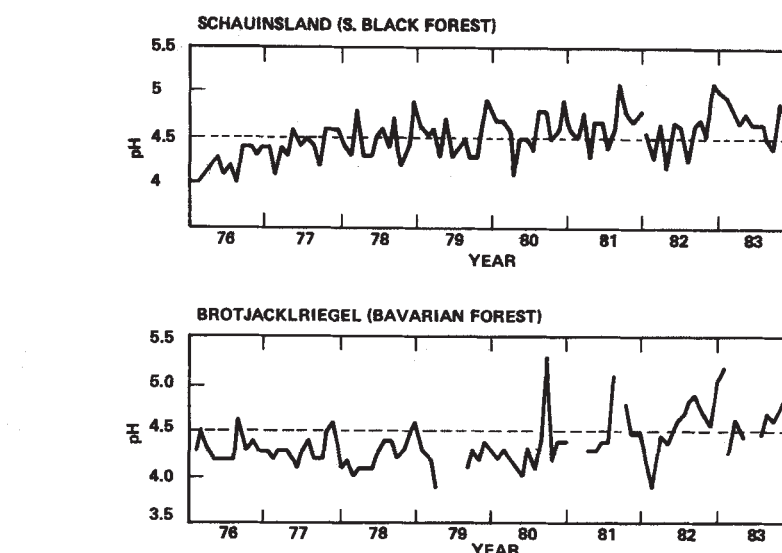


Fig. 2 Acidity of wet deposition (monthly means) in the Black Forest (Schauinsland) and the Bavarian Forest (Brotjackriegel)¹⁹.

damage to beech and oak has risen sharply since 1983 (Table 3). Although damage can now be found all over the country, the southern states of Bavaria and Baden-Württemberg still appear to suffer most.

Disputed results

The official damage data based on the inventories are, however, not undisputed. In particular, the methods of damage assessment have been heavily criticized^{15,16}. The criteria used for damage classification are unspecific but damage was, at least in 1983, ascribed to air pollution as a 'major or contributing cause'. There is now also a debate as to whether needle loss is a suitable criterion for damage classification. It has been suggested that needle loss up to 20% or 25%, currently resulting in a classification as 'slightly damaged', may in fact still be within the natural variation of healthy forests. The 1984 Swedish forest damage inventory, which is based on the German surveys, takes this into account, considering a needle loss of up to 20% as normal; the results of this survey are due to be published later this year.

Despite this controversy about assessment methods, the reliability of presented data and the comparability of the three inventories it is, however, undisputed that the decline has again increased and can

now probably be seen as the major environmental problem in Germany.

Potential causes

This widespread disease, now affecting a variety of species in stands with different characteristics could not be attributed to a known cause of previous dieback events. Factors such as extreme climatic conditions (such as frost, sudden changes in temperature, drought), insect or pathogen attack, unsuitable silvicultural practices (for example, selection of the wrong species or provenance), or local sources of pollution can not explain the geographical extent of damage and its sudden increase within a few years. The discussion of the potential cause or causes very soon centred around air pollutants, which are now widely believed to play a major role in the decline. 'Acid rain', a term which at the beginning of the 1980s was becoming increasingly popular in Germany (and which was often used as a synonym for air pollution in general), was initially blamed by the media¹⁷, and it was suggested, that SO₂ emissions in particular were the cause. This generalizing hypothesis has, however, in recent years given way to a more detailed discussion in which a number of potential causes have been suggested. It is now widely believed that the decline may be a complex disease, involving a number

Table 2 "New type" forest decline in 1983 and 1984 according to damage categories

Damage category	1983		1984	
	Area (ha × 10 ⁶)	Forest area (%)	Area (ha × 10 ⁶)	Forest area (%)
I	1.830	24.7	2.424	32.9
II	0.647	8.7	1.163	15.8
III + IV	0.076	1.0	0.111	1.5
I + II + III + IV	2.545	34.4	3.698	50.2

Categories defined as: I, slightly damaged; II, damaged; III, severely damaged; IV, dead. Total forest area, 7.4 × 10⁶ hectares.

of factors, and there is also the possibility that the widespread decline is a number of concurrent regional events with a common trigger.

Direct effect of acid

Direct effects of sulphuric and nitric acid are, at least within the scientific community, not generally considered to be primarily responsible for the damage. The rain in affected areas is not sufficiently acid to cause damage even to sensitive plants¹⁸, and there has been no increase in rain acidity in recent years in the Southern Black Forest or in the Bavarian forest, both areas severely affected by the decline (Fig. 2)¹⁹.

Soil acidification

The most widely accepted initial hypothesis was based on a suggestion that wet and dry acid deposition affects the soil by destroying its buffering system. This would lead to leaching of nutrients (for example Ca, Mg, K) and mobilization of toxic aluminium ions, which would damage the fine root system of the tree, eventually resulting in its death²⁰. The theory proposed that soil acidification is a natural process, but has in recent years been accelerated by increased acid input due to air pollutants. This ecosystem-orientated hypothesis of a soil-mediated effect is based on a comprehensive study of nutrient cycling in the Solling Mountains²¹ (North Germany; see Fig. 1), and the results were extrapolated to other areas. It has been extensively criticized²², mainly on grounds that damage occurs to trees on a variety of soils including those in calcareous areas with characteristics markedly different to those found in the Solling Mountains. The hypothesis cannot explain both the temporal and geographical development of the recent decline.

SO₂ and NO_x

High concentrations of SO₂ are known to cause damage to most plants including trees^{23,24}. Severe and large-scale damage to forests is reported from Poland and particularly Czechoslovakia (Erzgebirge, Riesengebirge), areas under the direct influence of high SO₂ concentrations from lignite combustion.

Only a comparatively small area in the FRG next to the East German and Czech borders (Fichtelgebirge) is exposed to similarly high SO₂ levels, with mean annual concentrations between 50 and 60 $\mu\text{g m}^{-3}$ and episodes of much higher concentrations (up to 1,500 $\mu\text{g m}^{-3}$)²⁵. Damage in this area has been attributed to the direct action of pollutants, and is similar to that observed in the Erzgebirge area in Czechoslovakia.

Direct effects of SO₂ and NO_x have not been proposed as the cause of damage for the majority of sites in the FRG. SO₂ levels are generally well below the limits suggested by the International Union of Forestry Research Organizations (IUFRO). They recommend a limit of 25 $\mu\text{g m}^{-3}$

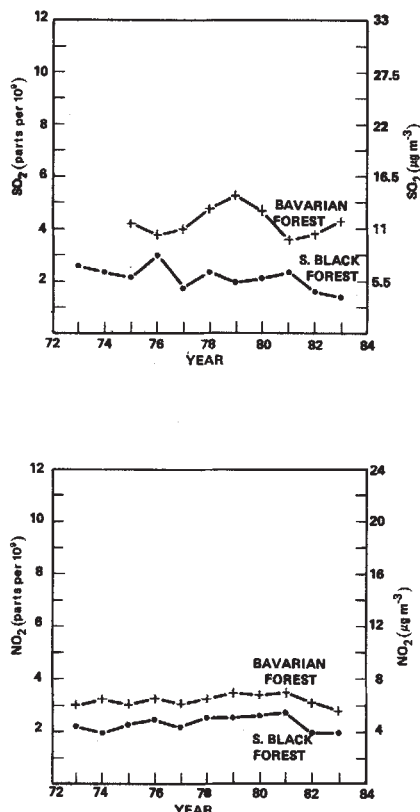


Fig. 3 Mean annual concentrations of SO₂ and NO₂ in the Black Forest (Schausinsland) and the Bavarian Forest (Brotjackriegel)¹⁹.

(annual mean), 50 $\mu\text{g m}^{-3}$ (average over 24 hours), and 75 $\mu\text{g m}^{-3}$ (97.5 percentile of 30-min values during the period of growth) in higher regions of mountains and in forests at extreme sites²⁶. The Schausinsland (Southern Black Forest) and the Brotjackriegel (Bavarian Forest) sites with forest damage, are both classified as 'clean air monitoring stations' by the German environmental protection agency (UBA). Annual mean SO₂ concentrations at the two sites during 1972–1983 were between 10 and 15 $\mu\text{g m}^{-3}$ (Bavarian Forest) and between 4 and 8 $\mu\text{g m}^{-3}$ (Black Forest), and annual mean NO₂ concentrations were below 8 $\mu\text{g m}^{-3}$ (Bavarian Forest) and 6 $\mu\text{g m}^{-3}$ (Black Forest) (Fig. 4)^{19,27}. A first indicator that SO₂ alone was unlikely to be the primary cause of damage was the prolific growth of lichens even in severely damaged forests.

Ozone

It was recently suggested that ozone (and possibly other photooxidants) may be in-

Table 3 Extent of 'new type' forest decline according to tree species

Species	Total area (ha × 10 ⁶)	Affected area (%) 1983	Affected area (%) 1984
Spruce	2.950	41	51
Fir	0.178	44	59
Pine	1.463	75	87
Beech	1.262	26	50
Oak	0.612	15	43
Others	0.940	17	31

involved in the decline²⁸, and the following mode of action was proposed²⁹: Exposure to elevated ozone levels damages cell membranes, resulting in an increased leaching of ions by acid fog and acid rain. Ozone also causes damage to chlorophyll, and thus reduction in carbohydrate production and root growth. This reduces the ability to compensate for leached nutrients by increased uptake. A similar hypothesis includes frost as another important factor^{30,31}.

Ozone levels in damaged forests in the FRG are often remarkably high^{29,32,33} and sometimes comparable to parts of the United States where ozone is known to have caused injury to trees³². Recent measurements show that daily mean ozone levels in 'clean air areas' of the Southern Black Forest (Schausinsland) and the Bavarian Forest (Brotjackriegel) are often above 100 $\mu\text{g m}^{-3}$ (refs 19,29) with annual mean concentration of the order of 80–110 $\mu\text{g m}^{-3}$ (Black Forest) and 60–90 $\mu\text{g m}^{-3}$ (Bavarian Forest).

There is, however, still a paucity of data, particularly from areas with extensive forest damage. There is also a shortage of data on long-term trends in ozone concentrations — only one data set gives a clear idea of the trend in natural background ozone concentrations in Central Europe over the past 20 years. Ozone levels at Arkona on the isle of Rügen in the Baltic Sea off the German Democratic Republic, an area not affected by local air pollution, increased by 60% between 1956 and 1977 (Fig. 4)³⁴. A similar trend has been reported for two other stations in forest areas of the German Democratic Republic³⁴, and it can be assumed for forest sites in the FRG and other neighbouring countries as well. This coincides with the steady increase of NO_x and hydrocarbon emissions from motor vehicles, which react with sunlight to produce ozone during transport over long

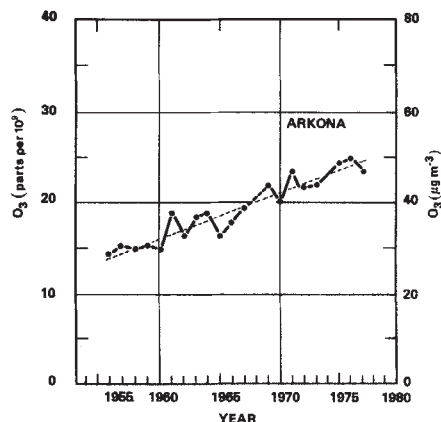


Fig. 4 Trend in mean annual ozone concentration at an unpolluted site in the German Democratic Republic (Rügen). Ozone was measured by bubbling air through KI solutions, so the data represent total oxidant. A filter was added in 1972 to remove interference from reducing gases such as SO₂ (redrawn from ref. 34).

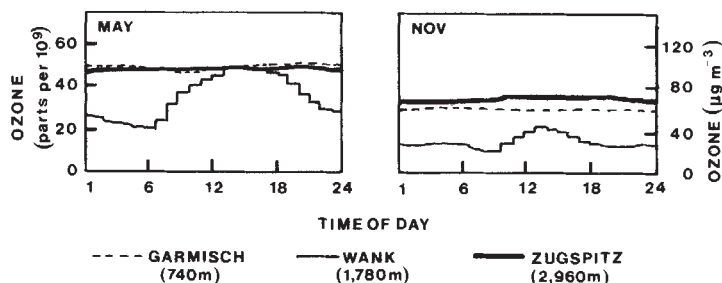


Fig. 5 Diurnal trends in ozone concentrations (1977-82) at three altitudes in the Garmisch-Partenkirchen Valley in the Bavarian Alps (Garmisch 740 m; Wank 1,780 m; Zugspitz 2,960 m). The site at Wank is close to the tree line (redrawn from ref. 35).

distances from urban areas. It has been suggested that the ozone would then affect remote areas, particularly at high altitudes, which are not under the influence of high levels of the 'classic' pollutants SO_2 and NO_x . Data from the Bavarian Alps (Garmisch-Partenkirchen) show low and steady ozone concentrations between 1977 and 1982 in a valley, and higher concentrations with a trend towards an increase at higher altitudes nearby³⁵. It was at higher altitudes of remote forest areas that damage was first noticed. What is perhaps most important, in this context, is the lack of the diurnal pattern, characteristic for urban areas, at higher altitudes where concentrations appear to remain remarkably steady for long periods (Fig. 5)³⁵.

Besides the involvement of ozone a second major factor has been suggested to interact with the oxidant; acid mist. A high incidence of fog is characteristic for higher altitudes in some damaged mountain areas in the FRG, with an annual mean of up to 226 days with periods of fog reported for a station in the Bavarian Forest³⁶. Data on the frequency, the duration, and the ion-concentration of fog collected in damage areas, however, are rare.

The photo-oxidant hypothesis is at present the most widely accepted explanation for the new-type forest decline. It is, however, still largely based on circumstantial evidence and needs further supporting data from forest areas as well as experimental verification. Experimental work using or exceeding concentrations of ozone believed to be common at areas with a high incidence of damage have so far failed to produce the chlorosis which is so characteristic of the decline in Norway spruce^{29,37}. There is also little known on the relative susceptibility of the species concerned, and conifers, which are thought to be more resistant than deciduous species, appear to be affected first³⁸. Another open question is the possibility of the quality of received radiation being involved. It cannot be ruled out that the higher proportion of ultraviolet radiation received by trees at higher altitudes could be involved in the degradation of chlorophyll in damaged needles. It will also be necessary to re-examine existing data on ambient air quality and to expand the network of air quality monitoring stations and monitored pollutants, preferably

by including sites in damaged areas.

Climate

Although there appears to be a consensus amongst scientists that air pollutants play a major and probably the key role in the decline, it has also been suggested that climatic factors may be involved. The series of dry and hot years experienced during the last decade has certainly affected tree vitality and resistance to pollutants and pathogen attack. Hot summers in the continental climate have often been followed by harsh winters, with temperatures below -15°C not an uncommon event. Drastic and sudden changes in temperature as on New Years Eve of 1978 when the temperature dropped by almost 20°C within hours may be equally important.

Pathogens

As the number of trees affected by fungal infections has markedly increased over the past few years, it has been suggested that pathogens play an important role, probably as a secondary cause of decline following a lowering of the resistance by pollution or climatic factors. Rehfuess and Rodenkirchen³⁹ report a comparatively high incidence of fungal infection (*Lophodermium* and other fungal pathogens) from forests

in Southern Germany in recent years.

There are also suggestions that the decline is an epidemiological rather than a pollutant-induced phenomenon⁴⁰. This theory suggests, that pathogens (probably mycoplasmas or rickettsia-like organisms) may be the cause of decline. This would explain the rather patchy occurrence of damage but this hypothesis is not yet supported by field or laboratory evidence.

Conclusions

The new-type decline is no longer confined to Germany. Damage has recently been reported from Switzerland (deciduous trees)¹⁰ and Sweden (spruce)⁴¹. Reports in the media suggest that similar damage can also be found in Italy, Austria and the Netherlands. The situation in Eastern European countries, where large-scale direct SO_2 injury is known to occur, is difficult to assess, as there are hardly any reports on the present state of the forests.

The alarming reports on the state of Germany's forests have aroused considerable and understandable emotion, sometimes overshadowing a realistic and critical discussion of the true extent and severity of the decline, of proposed causes and potential remedial measures. There is now, however, also an increased number of research projects under way. One would hope, that laboratory as well as field work will help to unravel the causes of the decline, as all proposed explanatory hypotheses, including the currently much favoured ozone-hypotheses, are still largely based on circumstantial evidence, requiring verification under realistic conditions. It would at present certainly be premature to conclude that a conclusive explanation for this apparently complex environmental problem is available. □

L. W. Blank is at the Gesellschaft für Strahlen- und Umweltforschung mbH, D-8042 Neuherberg (Munich), FRG, on a CEGB research fellowship.

1. Instructions for the 1984 Forest Damage Inventory (Federal Ministry for Food, Agriculture and Forestry, Bonn, 1984).
2. Wachter, A. J. *Pl. Dis. protect.* **85**, 361-381 (1978).
3. Malek, J. *Forstw. Cbl.* **100**, 170-174 (1981).
4. Seitschek, O. *Forstw. Cbl.* **100**, 138-148 (1981).
5. Wagner, F. *Forstw. Cbl.* **100**, 148-160 (1981).
6. Schröter, H. *Forstw. Cbl.* **100**, 161-168 (1981).
7. Säurehaltige Niederschläge (Verein Deutscher Ingenieure, Düsseldorf, 1983).
8. Jarowski, A. *Eur. J. For. path.* **12**, 143-159 (1982).
9. Moriando, F. & Covassi, F. *Forstw. Cbl.* **100**, 168-170 (1981).
10. Flückinger, W., Flückinger-Keller, H. & Braun, S. *Schweiz. Z. Forstwes.* **135**, 389-444 (1984).
11. Schröter, H. *Allg. Forstzeitschr.* **38**, 648-649 (1983).
12. Instructions for the 1983 Forest Damage Inventory (Federal Ministry for Food, Agriculture and Forestry, Bonn, 1983).
13. *Allg. Forstzeitschr.* **22** (Special Issue Forest Damage) (1983).
14. Lammel, R. *Allg. Forstzeitschr.* **14/15**, 340-344 (1984).
15. Ballach, H.-J. & Brandt, C.J. *Staub-Reinhalt. Luft* **43**, 448-452 (1983).
16. Führer, E., Halbwachs, G., Donaubauer, E., Pollanschütz, J. & Stefan, K. *Holz-Kurier* **39**, 13-14 (1983).
17. *Der Spiegel* 47/48/49 (1981).
18. Jacobson, J.S. *Phil. Trans. R. Soc.* **305**, 327-338 (1984).
19. Umweltbundesamt (UBA), Monatsberichte aus dem Meßnetz (1976-84).
20. Ulrich, B. in *Ecological Effects of Acid Deposition*, 221-231 (National Swedish Environment Protection Board, Solna, 1983).
21. Ulrich, B., Mayer, R. & Khanna, P.K. *Schriften Forstl. Fak. Univ. Göttingen*, **58** (1979).
22. *Gesamtverband des Deutschen Steinkohlenbergbaus, Saurer Regen und Forstschäden* 2nd edn (Essen, 1983).

23. Roberts, R.M., Darrall, N.M. & Lane, P. *Appl. Biol.* **9**, 1-142 (1983).
24. Materna, J. *Prace Vuhm.* **43**, 169-177 (1973).
25. Der Rat von Sachverständigen für Umweltfragen, Waldschäden und Luftverunreinigungen (Kohlhammer, Stuttgart/Mainz, 1983).
26. *IUFRO News* **25** (3) (1979).
27. Umweltbundesamt (UBA), Texte 33/1982 (1982).
28. Arndt, U., Seuffert, G. & Nobel, W. *Staub-Reinhalt. Luft* **42**, 243-247 (1982).
29. Prinz, B., Krause, G.H.M. & Stratmann, H. *LIS-Berichte* **28** (1982).
30. Bosch, C., Pfannkuch, E., Baum, U. & Rehfuess, K.E. *Forstw. Cbl.* **102**, 167-181 (1983).
31. Rehfuess, K.E. *Allg. Forstzeitschr.* **24**, 601-610 (1983).
32. Ashmore, M.R., Bell, J.N.B. & Rutter, A.J. *Forest Damage in West Germany and the Role of Ozone* (Imperial College, London, 1984).
33. Guderian, R., Tingey, D.T. & Rabe, R. in *Umweltbundesamt Berichte* 5/83 (ed. Guderian, R.) 205-429 (1983).
34. Warmbt, W. *Zeitschr. Met.* **29**, 24-31 (1978).
35. Reiter, R. *Bayerisches Staatsministerium für Landesentwicklung und Umweltfragen, Materialien* **28** (1983).
36. Noack, E.M. *Nationalpark Bayerischer Wald*, 5 (Bayerisches Staatsministerium für Ernährung, Landwirtschaft und Forsten, 1979).
37. Skeffington, R.A. & Roberts, T.M. *Oecologia (Berlin)* **65**, 201-206 (1985).
38. Davis, D.D. & Raymond, G.W. *EPA Report EPA-600/3-76-102* (1976).
39. Rehfuess, K.E. & Rodenkirchen, H. *Forstw. Cbl.* **103**, 248-262 (1984).
40. Kandler, O. *Naturwiss. Rundschau* **11**, 488-490 (1983).
41. Kvist, K. & Barklund, P. *Forstw. Cbl.* **103**, 74-82 (1984).

Recalculating interatomic forces

The van der Waals force between atoms and molecules is generally taken to be inversely proportional to the inverse sixth power of the distance, but positronium atoms may interact differently.

ALTHOUGH the van der Waals force has been part of classical physics for the best part of a century, since the recognition that the departure of the behaviour of real gases from the perfect gas laws points to a short-range attractive force between atoms and molecules, there is no classical explanation of the effect. Provided that the interacting species have no permanent dipole moment (when the van der Waals force is negligible), the best that can be done is to suppose that atoms and molecules are attracted to each other as a consequence of their polarizability; each becomes a little dipole under the influence of the other.

Classically, it is easy to see that this force has a short range in the sense that it will be proportional to a large power of the reciprocal distance R between the interacting entities (for the force due to an induced dipole is already proportional to $1/R^3$), but two interacting atoms which mutually induce a symmetrical pair of dipoles will be found to have a mutual energy greater than they began with, corresponding to repulsion, and which classically will not happen. That is why the van der Waals force has been regarded as essentially a quantum phenomenon at least since F. London's successful account of it half a century ago. Now, even that has been refined by means of an elegant calculation due to J.R. Manson and R.H. Ritchie (*Phys. Rev. Lett.* **54**, 785; 1985). The outcome could be interesting.

The simplest way of calculating the van der Waals force gives a reasonable result, but is now shown to be subtly too simple. For definiteness, suppose that the two interacting atoms are two hydrogen-like atoms whose nuclei are separated by a distance R . To a first approximation, the single electrons of each of them are described by a set of hydrogen-like wavefunctions centred on points separated by a distance R . The more realistic next approximation then consists in the addition to the total energy of the system of the terms representing the interaction between the pair of nuclei and the pair of electrons (both positive, denoting repulsion) and between each nucleus and the other electron (negative, for attraction).

Practitioners of wave mechanics will take this kind of problem in their stride. The extra interaction energy is supposed small compared with the interaction between each nucleus and its "own" electron, so textbook perturbation theory applies. The complete set of wavefunctions of each atom enters into some linear combination of the

wavefunctions corresponding to all of its quantum states. London remarked that geometrical symmetry ensures that the first-order perturbation calculation is zero. But second-order perturbation theory yields a plausible result: an attractive force between atoms proportional to $1/R^6$.

At its simplest, perturbation theory is a way of calculating the energy of a system that differs to only a small extent from one that can be solved exactly. For the calculation of van der Waals force between two hydrogen-like atoms, for example, the behaviour of each electron under the influence of its own nucleus is exactly calculable, and the result is the familiar infinite set of discrete energy levels converging on the ionization energy of the atom. The energy of the combined system of two atoms close together is then approximately calculable from the terms representing the interaction between the atoms and the wavefunctions corresponding to the original unperturbed system, the product of two one-electron wavefunctions centred on the positions of the two nuclei separated by a specific distance.

Second-order calculations give a familiar result. The energy of the interacting system differs from that in which the interaction is ignored by an amount expressed as an infinite series in which each term represents the square of the probability that the interaction energy will induce a transition from the original state to another but weighted by the reciprocal of the energy difference between the states. Straightforward calculation along these lines recovers the London result that the energy of the combined system is decreased by an amount proportional to $1/R^6$, entirely appropriate for the short-range van der Waals force.

But the argument is incomplete. Implicit in the calculation is the assumption that the pair of interacting atoms is static, with the two nuclei fixed in space. Yet the calculation itself suggests that the interaction is responsible for an attractive force between the pair of atoms which in the absence of specified constraints, should be sufficient to make the configuration of the system time-dependent.

The circumstances resemble those familiar in the calculation of the properties of molecules, where it is customary to calculate electronic states on the assumption that the atomic nuclei are fixed in space, and then separately to calculate such things as the vibrational frequencies of the

molecule, which entails allowing that the nuclei can move. The justification of this is the Born-Oppenheimer approximation, which is usually sustained by the observation that the characteristic frequencies of electronic motions are much greater than those with which atomic nuclei move. One day some lucky graduate student will find a supervisor to back sober up-to-date investigation of the circumstances under which the approximation does not apply.

Meanwhile, Manson and Ritchie have provided an intriguing example with which to begin, the van der Waals interaction between two positronium atoms, the metastable atoms consisting of a positron and an electron, the least massive of all hydrogen-like atoms. The starting-point for the calculation is a description of the unperturbed state of the system which allows that each of the interacting atoms may be in motion (so the wavefunctions are products of two atomic wavefunctions and two momentum wavefunctions, or plane waves). What Manson and Ritchie have done is to calculate the van der Waals force between two atoms whose initial states are described in this way.

First, there is a matter of interpretation, which goes back to London. The terms in the series representing the second-order perturbation energy, which are products of the probability that the interaction energy will cause transitions between electronic states, may be regarded as representing processes in which a virtual photon is allowed to excite the system in a defined way and then be emitted again, to be absorbed where it began. But, in such a process, there must be not merely a transfer of energy but of momentum — the atoms concerned must recoil. Manson and Ritchie have allowed for recoil in the calculation of the forces between positronium atoms. So the $1/R^6$ behaviour of the van der Waals force is modified, by the effects of recoil, to one that tends to $1/R^4$ at small separations between the atoms.

The chief interest in this work will be the neatness of the calculation. For the interaction between positronium atoms, the departure from $1/R^6$ behaviour should be recognizable out to a distance of four atomic units, and may thus be measurable. So should be departures from London's result for the interaction between positronium and other atoms. Meanwhile, the calculation is a telling reminder that even the best-tried algorithms should be constantly reassessed.

John Maddox

Synthetic fuels

From lignin to coal in a year

from John Larsen

SEVERAL million years and a minor imperfection in the carbon cycle have given us coal, yet Winans and his colleagues at Argonne National Laboratory have taken less than one year to prepare a thoroughly characterized synthetic coal¹. The material they produce is indistinguishable from the real thing by all the techniques so far applied to it and its synthesis raises many interesting questions in coal chemistry.

Coals are extraordinarily complex mixtures, which Neavel has likened to fruit-cakes², with the various components originating from different parts of the plants from which the coals are formed. Coal's organic constituents, which range in size from methane to macromolecular gels of effectively infinite molecular weight, are mixed throughout with a variety of inorganic materials, principally clays. Determining the structure of coal has been difficult because the normal techniques of organic chemistry are not well suited to complex macromolecular mixtures.

As is usually the case, instrumental advances have been responsible for much of the recent progress in understanding coal structures. The resurgence, in semi-quantitative form, of the concept of coal as a three-dimensionally cross-linked macromolecular structure — originally developed by van Krevelen³ — came just in time to support new and improved spectroscopic techniques⁴; the switch from grating IR instruments to Fourier-transform IR spectrometers not only added the kind of sensitivity that is so valuable with opaque samples, but also enabled spectra to be subtracted from one another, an enormous aid when working with complex mixtures. The recent availability of high-resolution nuclear magnetic resonance (NMR) spectra of solids is also being vigorously exploited and has greatly extended our ability to follow structural changes. This technique is still troubled by the 'invisibility' to NMR of many of the carbon atoms in coal, but all the indications are that visible carbon atoms are representative of the whole sample. New chemical methods, mostly better oxidative⁵ and reductive⁶ degradation techniques, are also contributing to structural information on coal. But these contributions have added little to our understanding of the process by which coal is formed. It is in this respect that the Argonne group's description of a convenient preparation of man-made coal is likely to be most important; already it has raised the possibility that natural coal production is a catalytic process.

The synthetic coal is produced by warming lignins (highly aromatic molecular components of woody tissue) at 150°C for a few months in the presence of twice as

much montmorillonite clay, which seems to serve an acid-catalytic role. Fourier-transform IR, NMR, electron-spin resonance, gas chromatography, mass spectrometry and oxidative degradation were used to analyse the product. The changes that were observed are similar to those that occur in natural coal production. Lignin heated without clay did not produce coal, and heating cellulose (the other portion of woody tissue), with or without clay, also failed to produce coal, perhaps settling the controversy over the role of cellulose in coal formation. More surprisingly, the clays cause fatty acids to form a coal component called alginite, which is thought to derive naturally from the remains of algae that contain fatty acids, as an important lipid constituent.

The most thorough previous attempt to produce coal artificially was a thirty-year experiment initiated at the US Bureau of Mines by R.A. Friedel^{7,8}. This experiment, which outlived the laboratory that started it, consisted of pyrolysis at 200°C of several possible coal precursors, including pine wood and lignite, a very young brown coal with high oxygen content. As judged by NMR, electron-spin resonance, IR spectra and elemental analysis, in only eleven years lignite was altered to a material similar to a much older coal and the pine wood formed material similar to a sub-bituminous coal. Interestingly, pyrolysis of pine wood at 300°C for one hour produced coal-like materials, which contrasts with

Winan's observation using lignin as a starting material. According to H. Retcofsky, who is completing a study of the Bureau of Mines's samples, pyrolysis for eleven years at 200°C is roughly equivalent to one hour at 290°C.

The Argonne work raises many questions, particularly concerning the relevance of acid catalysis using large amounts of clay. Real coals often contain only a few per cent of coal clays. Is their production catalysed by clays? What is the mechanism by which acid catalysis is extended into the solid from the clay surface? Is this mixture of solids mobile? If so, will time alone allow the few per cent of clay in natural coal to convert the entire mass? What is the pH dependence of this acid-catalysed process? These questions will surely be addressed. The production of artificial coal will open a new era of the study of coal formation by allowing the direct experimental test of hypotheses. The preparation of isotopically-labelled coals for spectroscopic and other studies, is already underway and will be welcomed by those trying to unravel coal reactions. □

1. Hayatsu, R., McBeth, R. L., Scott, R. G., Botto, R. E. & Winans, R. E. *Org. Geochem.* **6**, 463 (1984).
2. Neavel, R. C. In *Chemistry of Coal Utilization* Suppl. Vol. (ed. Elliott, M. A.) Ch.3 (John Wiley, New York, 1981).
3. van Krevelen, D. W. *Coal* (Elsevier, New York, 1981).
4. Karr, C. Jr. *Analytical Methods for Coal and Coal Products* (Academic, New York, 1978).
5. Hayatsu, R., Scott, R. G. & Winans, R. E. in *Oxidation in Organic Chemistry* Part D (ed. Trahanovsky, W. S.) (Academic, New York, 1982).
6. Stock, L. M. in *Coal Science* Vol 1 (eds. Gorbaty, M. L. Larsen, J. W. & Wender, I. (Academic, New York, 1982).
7. Friedel, R. A., Queiser, J. A. & Retcofsky, H. L. *J. phys. Chem.* **74**, 908 (1970).
8. Friedel, R. A. & Retcofsky, H. L. in *Spectrometry of Fuels* (ed. Friedel, R. A.) Ch. 5 (Plenum, New York 1970).

John W. Larsen is in the Department of Chemistry, Lehigh University, Bethlehem, Pennsylvania 18015, USA.

Early mathematical wheelwork

Byzantine calendrical gearing

from Francis Maddison

FOUR fragments of a sundial incorporating a geared calendar (Fig. 1), inscribed in Greek and probably dating from the late fifth or the early sixth century of our era, document a gap in the history of precision gearing as yet unfilled by literary evidence. The fragments are extensively described and discussed by J.V. Field and M.T. Wright in a forthcoming paper¹; and at an exhibition* that opens today in London's Science Museum, the fragments are set in historical relationship to the very few surviving devices with precision gearing from the last century BC to the beginning of the fourteenth century.

It may still commonly be thought that gears with precisely determined numbers of teeth were first used in mediaeval clocks,

but an early discovery of underwater archaeology disproved that view. In 1901, the cargo of a ship which sank around 80 BC near the island of Antikythera in the Aegean was found to contain a corroded mass of bronze gear-wheels and fragments of bronze plates with Greek inscriptions. The late Derek de Solla Price showed that these remains had formed part of a complex calendrical computing device, a fact which fitted uneasily into the traditional view of a Hellenistic society little interested in technology². The main input into the box containing the mechanism was by a crank which rotated a contrate wheel to turn a main wheel, one revolution of which represented a solar year. Outputs seem to have included a function of the Metonic cycle (using a differential), the relative positions of the Sun and Moon, and the risings and settings of certain stars throughout

*'Early Gearing: Geared Mechanisms in the Ancient and Medieval World', from 28 March 1985. Exhibition catalogue from Science Museum, London SW7 2DD, UK.

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

Fig. 1 The four fragments (left) and a reconstruction (right) of a Byzantine sundial-calendar with Greek inscriptions and probably made in AD 479-565. The large disk has a diameter of 135 mm. Reconstruction by M.T. Wright. (Courtesy Trustees of the Science Museum).

the year. The Science Museum exhibition includes a model of the Antikythera machine (lent by the Smithsonian Institution, Washington).

Price related the triangular-toothed gear-wheels of this mechanism to a Hellenistic tradition of automata and mechanical toys, applied to an astronomical water-clock tradition, as recorded by Vitruvius around 25 BC. He pursued the tradition further, by way of Islamic interest in automata and water-clocks with opening windows, spherical weights falling into gongs, and moving human figures, to the mediaeval European astronomical clocks, with their mathematical modelling of the motions of the celestial bodies (as on the planetary clock of Giovanni de' Dondi, completed in 1364) and astrolabe dials and jacks (like the clock of 1410 on the Old Town Hall in Prague)³⁻⁵. This is an involved and complicated story, fascinating in its implications for motivation and cross-cultural transmission, but the gaps in the sequence are wide and the pursuit of detail a difficult task.

Chronologically, the history of mathematical gearing has been devoid of surviving artefacts or literary description from the Antikythera mechanism until about AD 1000, when the eminent Islamic scientist al-Bīrūnī wrote a treatise on the different types of astrolabe, and included an account of a geared calendar. The Science Museum exhibition includes a reproduction of al-Bīrūnī's text and an astrolabe (made in AD 1221-2 by Muhammad the son of Abū Bakr, the needle-maker of Isfahān, and borrowed from the Museum of the History of Science, Oxford) which includes just such a geared calendar (Fig. 2), though with slightly different gear-ratios. On the back-plate are apertures showing (in the Arabic alphabet, used numerically) the age of the Moon, and its phase by the rotation of a silvered and blackened disc; furthermore, the relative positions of the Sun and Moon are indicated by inlaid gold and silver

points on concentric rings within a scale of the signs of the zodiac. A gear-train links the manually-turned discs giving the data, confirming al-Bīrūnī's remark, two hundred years earlier, that the wheels in his calendar were like those fitted to the backs of astrolabes.

The fragments acquired by the Science Museum, regrettably without any provenance beyond the vendor's statement that they were bought in the Lebanon, fall chronologically midway between the Antikythera mechanism and the al-Bīrūnī calendar. A detailed metallurgical, technological and historical study of the fragments¹, together with a new translation and commentary on al-Bīrūnī's description of the geared calendar⁶, show that the fragments were part of a geared calendar, the gear-ratios of which correspond exactly with those given by al-Bīrūnī, associated in this case not with an astrolabe but with a portable vertical disk sundial of a type well-known from late antiquity. The Greek in-

scriptions and the date of manufacture attributed to the fragments — before AD 650, probably AD 479-565 — indicate a source for the al-Bīrūnī calendar, and push back its origins by at least 500 years. Among the fragments is the front plate which bears a scale for adjustment to the latitude of the sundial, scales of solar declination for adjusting the gnomon (missing) which bore the hour-lines, and a table of the latitudes of various places (including Constantinople, Alexandria, Athens, Sicily, and Rome). It also has a circular scale of the days of the week (indicated by pictorial representations of the 'rulers' of the day), over which moved a pointer linked to the calendar. The back plate and some wheelwork are missing, but a ratchet survives, suggesting that the calendar mechanism might have been 'clicked on' each day, to provide the date for setting the gnomon. Field and Wright discuss the possible use of the calendrical mechanism to convert sundial readings by moonlight to solar time, and to predict eclipses. Remarkably, geared calendars of this type were still in use at the end of the sixteenth century.

Because of their age, the new fragments impose a revaluation of commonly held views of Byzantine science and technology. The exhibition of which they form a part covers an important and exciting theme — fourteen centuries of precision mathematical gearing for computation before the end of the European mediaeval period. □

1. Field, J.V. & Wright, M.T. *Annals of Science* (in the press).
2. Price, D.J. de S. *Scient. Am.* June 1959; *Gears from the Greeks* (Science History Publications, New York, 1974; reprinted from *Trans. Am. Phil. Soc.* n.s. 64, 1; 1974).
3. Hill, D. *A History of Engineering in Classical and Medieval Times* 183 (Croom Helm, London & Sydney, 1984).
4. King, H.C. & Millburn, J.R. *Geared to the Stars. The Evolution of Planetariums, Orreries and Astronomical Clocks* Ch. 1-4 (Toronto U.P., Toronto and Hilger, Bristol, 1978).
5. Bedini, S.A. & Maddison, F.R. *Mechanical Universe. The Astrarium of Giovanni de' Dondi* (Museum of History and Technology, Washington 1966; reprinted from *Trans. Am. Phil. Soc.*, n.s. 56, 1; 1966).
6. Hill, D.R. *Annals of Science* (in the press).

Francis Maddison is Curator of the Museum of History and Science, Oxford OX1 3AZ, UK.

Fig. 2 Calendrical gearing (left) and back-plate (right) of a Persian astrolabe made in AD 1221-2 by Muhammed the son of Abū Bakr, the needle-maker of Isfahān. (Courtesy Museum of the History of Science, Oxford.)

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

Immunogenetics

Lymphocyte development genes and immunodeficiency disease

from S. Darling and P. Goodfellow

UNLIKE AIDS, some human immunodeficiency diseases are inherited rather than acquired. Several of these genetic diseases are linked to X-chromosome loci and are responsible for catastrophic illness^{1,2}. As neither the immunoglobulin genes³ nor the T-cell receptor genes are themselves sex linked^{4,5}, it has been suggested that the X-linked disease loci might include regulatory genes responsible for lymphocyte development. This hypothesis is supported by two papers in this issue^{7,8}, which report the isolation of an X-linked sequence that is developmentally expressed in lymphocytes and is aberrantly expressed in a mouse model for X-linked immune deficiency.

CBA/N mice have an X-linked mutation, *xid* (reviewed in ref. 9), which renders them incapable of producing antibodies to soluble polysaccharides. Their immunodeficiency is caused by a B-cell defect and the mice seem to lack a specific subpopulation of mature B cells. In humans, a similar defect in antibody production is present in the X-linked Wiskott-Aldrich syndrome¹⁰, which, unlike the mouse disease, is accompanied by defects in T-cell and platelet function. Arguing that the mouse *xid* genes affected in X-linked immunodeficiencies would be expressed in lymphocytes⁷, Cohen *et al.* sought *xid*-related messenger RNA by the 'subtraction' cloning technology developed for isolating the T-cell receptor genes^{11,12}. Of the 15 lymphocyte-specific cDNA clones thus prepared, one clone, which is apparently T-cell specific, recognized an X-linked gene family that might contain as many as 15-20 members. These genes have been named *XLR* (X-linked, lymphocyte-regulated)⁷.

Two lines of circumstantial evidence suggest that the *XLR* genes are directly related to the *xid* locus. First, genetic studies with congenic strains of mice show there to be very close linkage between the *xid* locus and the *XLR* locus. Second, detailed expression studies detect *XLR*-derived

mRNA not only in T cells but also in plasma cells and other B cells at late stages of differentiation. Unlike plasmacytomas derived from normal mice, plasmacytomas from *xid* mutant mice do not contain *XLR* transcripts⁸. Independent evidence has implicated mature B cells as the target tissue for the *xid* locus⁹. So, either the *xid* locus is regulating the *XLR* locus or the two loci are identical. Final proof of identity will require demonstration of an alteration in an expressed *XLR* gene that is the result of the *xid* mutation.

The X-chromosome of eutherian mam-

mals has been conserved during evolution¹³ and the presence of an *XLR* locus in humans can be predicted confidently. It will be of obvious interest to study *XLR* expression in Wiskott-Aldrich syndrome and in the other human X-linked, immunodeficiency syndromes. Even if mutation at the *XLR* locus is not directly responsible for disease, this gene family is certain to be studied in detail. With a developmentally-regulated expression that is apparently restricted to lymphocytes, and with the possibility that different members of the gene family are transcribed at different times, the *XLR* genes may well be responsible for regulating lymphocyte development and are therefore bound to be extensively examined in the coming months □

S. Darling and P. Goodfellow are at the Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK.

Molecular biology

Coordination of sequence data

from Arthur M. Lesk

MANY challenges are presented by the very rapid growth of DNA and protein sequence information, the opportunities arising from advances in computer data-base management facilities, and the variety and importance of the applications of this knowledge. Not least is the challenge of coordinating the various data-banking projects.

The main archives of nucleic acid sequences, at the European Molecular Biology Laboratory Library and GenBank (a collaborative effort between Los Alamos National Laboratory and Bolt, Beranek and Newman), now contain approximately 3×10^6 bases, about half of the total of published sequences. It is estimated that another 5×10^5 bases are currently being published every six months, and the process is still accelerating.

The major archive of amino-acid sequences of proteins (now often derived from gene sequences) is the Protein Information Resource, based at the National Biomedical Research Foundation in Georgetown, Virginia, now under the direction of W.C. Barker. It contains over 3,000 entries, comprising over 6×10^5 amino-acid residues. R.F. Doolittle of the University of California at San Diego, and S. Sakakibara of the Protein Research Foundation, Osaka, Japan, also maintain protein-sequence data banks. In addition, numerous specialized collections exist, such as the 'Sequences of Proteins of Immunological Interest', published by the US National Institutes of Health, and the International Haemoglobin Information Center, which is directed by R.N. Wrightstone of the Medical College of Georgia.

The Protein Structure Data Bank at Brookhaven National Laboratory, directed

by T.F. Koetzle, contains, in its latest release, 253 sets of atomic coordinates of proteins and nucleic acids, almost all derived from X-ray crystallography.

Current activities fall into four categories. First, there is data acquisition by an archive. The basic chain of events at present is: publication; surveillance of the literature and extraction of published data by the archiving project; quality control; editing, formatting and addition to data base. Second, the archived information is distributed on magnetic tape or, if only small amounts of information are involved, over computer networks. Third, there is information retrieval, dependent on the development of computer program systems as interfaces between archived information and research projects. Finally, there are applications — for example, the search of a data base for sequences similar to the one that has been newly determined.

Those of us whose activities primarily involve applications depend, ever more crucially, on the quality, completeness and accessibility of the archives. Despite the stake of the scientific community at large in these resources, individual projects have so far borne the responsibility for collecting, maintaining and distributing the data. In contrast, many groups all over the world are developing software for information retrieval and research applications in molecular biology. To the extent that the computer programs are well described and readily distributed, they are subject to ordinary processes of natural selection from which, one may presume, the most effective will ultimately emerge in common use.

How can new generations of molecular biologists and new generations of com-

1. Waldman, T.A., Strober, W. & Blaes, R.M. in *Clinical Immunology* Vol. 1 314 (W.B. Saunders, Philadelphia, 1980).
2. McKusick, V.A. *Mendelian Inheritance in Man* (John Hopkins University Press, 1983).
3. *Human Gene Mapping 7*; *Cytogenet. Cell Genet.* 37, 1 (1984).
4. Collins, M.K., *et al.* *EMBO J.* 3, 2347 (1984).
5. Collins, M.K. *et al.* *Nature* (in the press).
6. Stinson, A. *et al.* (submitted).
7. Cohen, D.I. *et al.* *Nature* 314, 369 (1985).
8. Cohen, D.I., Steinberg, A.D., Paul, W.E. & Davis, M.M. *Nature* 314, 372 (1985).
9. Scher, I. *Adv. Immunol.* 33, 1 (1982).
10. Cooper, M.D., Chae, H.P., Lowman, J.T., Krivit, W. & Good, R.A. *Am. J. Med.* 44, 499 (1968).
11. Hedrick, S.M., Cohen, D.I., Nielsen, E.A. & Davis, M.M. *Nature* 308, 149 (1984).
12. Davis, M.M. *et al.* *Proc. natn. Acad. Sci. U.S.A.* 81, 2149 (1984).
13. Ohno, S. *Monograph Endocrinol.* 1 (Springer, 1967).

puters interact most effectively? Certain algorithms and computer programs are already mature and applicable directly to the data bases that are increasing so fast in scope. The introduction of very large, fast computers and the design of special-purpose chips will have several effects. Certain tasks, for which computational power is the limiting resource, will for the first time become feasible. The prediction of protein structures from sequences may fall into this category, although there is considerable pessimism about the adequacy of the models on which such calculations have so far been based. Faster computers will also enable some tasks, now possible but slow, to be performed interactively. This will stimulate changes in the design of algorithms and user interfaces.

The intimate combination of graphical and numerical approaches to computational molecular biology will continue to be essential. A scientist analysing a protein or nucleic acid structure will want to ask a variety of questions. The computer's response may take the form of a picture, or text, or both. When possible, the program will respond at conversational speed, to establish a dialogue between user and program. Which tasks are interactive and which must be 'spun off' into batch queues must depend on the hardware configuration.

Until now, many programs have been designed to answer descriptive questions about protein sequences or structures. To design systems that address more complicated questions, such as "What if...?" or "What is the relationship between...?", the user must be able to perform computational experiments that modify the structures. Such questions will require more powerful interactive and manipulative tools, and are likely to involve the development of expert systems.

A major problem in creating programs for a worldwide community of users is the design of supple software, adaptable to the needs of different users. A very general concept suggested some time ago may provide a useful approach. This is the 'virtual data base' — an information-access system that is self-modifiable, under user control. Each session of each user will produce an 'active state' in which certain working files of structures have been created, identified and linked, and certain groupings of elementary operations defined to create a working set of higher-level commands and procedures. It will be useful to be able to save, restore and manipulate the 'active state of the data base', or to define a 'start-up procedure' to establish, at the beginning of any session, a state tailored to the individual investigator or even to the particular project.

The need to establish a mechanism for coordination stems not from any dissatisfaction with the operation of information management projects currently in existence, but from the fear that Malthusian growth will saturate the

channels of data acquisition, distribution, and utilization. To meet the need, CODATA (the Committee on Data of the International Congress of Scientific Unions) has established a Task Group on coordination among data banks. This group, chaired by Professor B. Keil of the Institut Pasteur, and comprising representatives from Europe, North America and Japan, has been charged with enhancing international cooperation in archiving, distribution and utilization of information related to protein sequences.

General problems that the CODATA task group plan to address include:

- (1) Maintaining directories of protein sequence data bases* — including the special features of their contents or support facilities, addresses of their directors, and availability — and of computer programs for data retrieval and applications.
- (2) Encouraging the establishment of guidelines and use of standardized nomenclature, and the development and general acceptance of common formats for data exchange.
- (3) Establishing links between protein sequence data banks and data banks containing related information, such as nucleic acid sequences or protein structures.

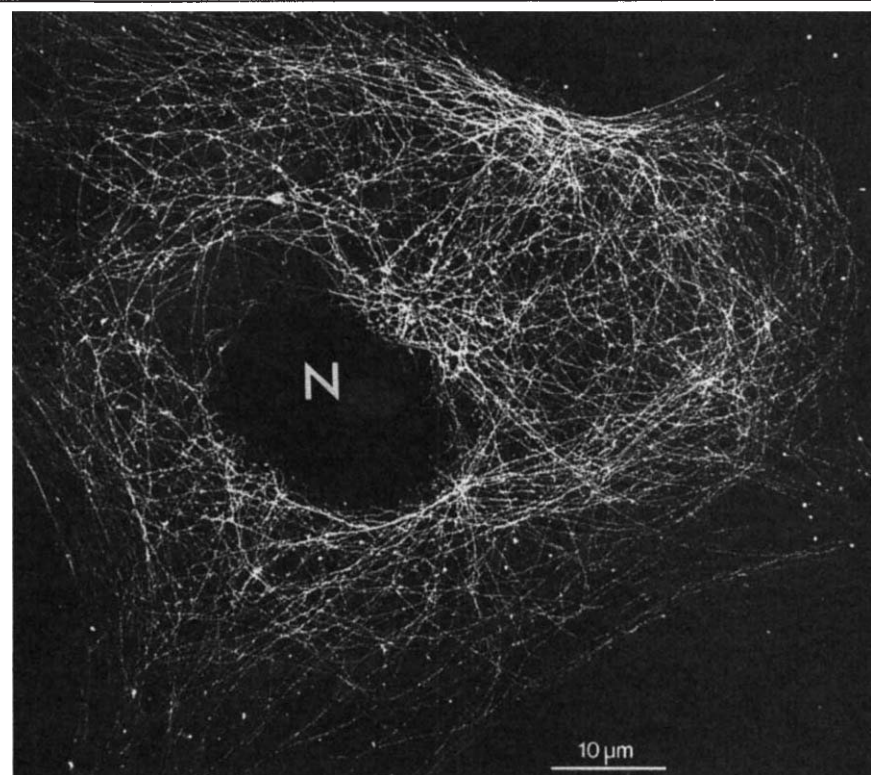
*The committee would be grateful if individuals who have assembled such data banks would write to the chairman, so that the catalogue may be as complete as possible.

(4) Fostering coordination of efforts to avoid duplication and to maximize the effectiveness of the work of different groups in different countries; for example, by identifying gaps in current programs and encouraging the development of specialized data bases when appropriate.

(5) Guiding the design of distributed material to enhance the general utility of the data banks, taking into account the needs of scientists without sophisticated familiarity with computer programming or those with access to limited computer resources. This may include the development of introductory tutorial documents for new users, or sponsoring training courses as satellite sessions of scientific meetings.

The effective coordination of the efforts of one scientist and one computer working together on a single project is primarily a technical one. The effective coordination of the efforts of many scientists, working separately on different projects, requires the personal touch as well. It is hoped that the CODATA Task Group will be able to pay due attention to both sorts of problems, and thus be able to channel the force of the information explosion in molecular biology into even more productive directions. □

Arthur M. Lesk is at the Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK and is a member of the CODATA Task Group.



Microtubule network of an epithelial cell visualized by immunophotoelectron microscopy. N is the nucleus. The technique combines photoelectron imaging with immunofluorescence. For this example, the cell was first treated with a monoclonal antibody to microtubules, then with a fluorescently-labelled antibody to the first antibody, and finally with a colloidal gold-labelled antibody to the second antibody. The second stage was used to enable the technique to be compared with immunofluorescence microscopy, which has a resolution of 200 nm, whereas the resolution of immunophotoelectron microscopy is 10–20 nm. From Birrell, G.B., Habliston, B.L., Nadakavularen, K.K. & Griffith, O.H. *Proc. natn. Acad. Sci. U.S.A.* 82, 109; 1985).

Astronomy

Supernovae and the distance scale

from Sidney van den Bergh

IN SPITE of fifty years of observational effort, the Hubble constant, which measures the size (and somewhat less directly the age) of the Universe, remains poorly determined. That is so because galaxies are extremely distant and so the 'standard candles' within them are exceedingly faint. The most luminous known standard candles are supernovae of type I (SNI) at maximum light. On page 337 of this issue, W.D. Arnett, D. Branch and J.C. Wheeler suggest that it may be possible to calibrate the maximum luminosity of SNI directly from nuclear physics. This approach bypasses the traditional and error-prone 'distance ladder' technique for the determination of the extra-galactic distance scale.

It is now generally believed that the precursors of SNI are mass-accreting carbon/oxygen white dwarfs in close binary systems. The C-O nuclear fuel in these objects will ignite if mass transfer from a binary companion pushes the total mass of the white dwarf above about 1.4 solar masses (the Chandrasekhar limit). Nuclear incineration of C-O to radioactive ^{56}Ni in an exploding white dwarf will produce 1.4×10^{51} erg per solar mass. Most of this energy ends up as kinetic energy of matter ejected during the supernova explosion.

The optical luminosity of SNI is believed to result from the trapping and thermalization of the γ rays and positrons emitted by the decay of ^{56}Ni through ^{56}Co to ^{56}Fe . Detailed hydrodynamical calculations have indicated that a C-O white dwarf that is pushed over the Chandrasekhar limit will produce 0.4–1.4 solar masses of ^{56}Ni . Based on these calculations and other evidence, the best estimate of Arnett *et al.* is that a SNI will produce 0.6 solar masses of ^{56}Ni and that the decay of one solar mass of ^{56}Ni would produce a maximum supernova luminosity of $(2.2 \pm 0.2) \times 10^{43}$ erg s^{-1} . They then deduce, from knowledge of supernova spectra, that the decay of 0.6 solar masses of ^{56}Ni will produce a supernova with a maximum blue magnitude, $M_B(\text{max})$, of -19.5 , with limits of -20.4 and -19.1 , corresponding to 1.4 and 0.4 solar masses of ^{56}Ni .

For SNI that have been observed to occur in elliptical and lenticular galaxies (which are generally dust-free) with redshifts larger than $3,000 \text{ km s}^{-1}$ (for which random motions of individual galaxies can be neglected), Arnett *et al.* find that $M_B(\text{max}) = -18.4 + 5 \log (H/100)$. Substitution of their calculated value of $M_B(\text{max})$ into this relation yields $H = 59 \text{ km s}^{-1} \text{ Mpc}^{-1}$, with upper and lower limits of 73 and $39 \text{ km s}^{-1} \text{ Mpc}^{-1}$.

This result places Arnett *et al.* firmly on the side of those who find that the Hubble

constant is small enough to accommodate the age of the Universe derived from evolutionary age models of globular cluster stars — about 1.8×10^{10} years. Those who favour larger values of the Hubble constant and smaller ages of the Universe will, no doubt, bring forward four arguments. First, incineration of a carbon/oxygen white dwarf might, in fact, produce less than 0.4 solar masses of nickel. Second, calculation of the maximum optical luminosity of SNI involves rather complex radiative transfer calculations in a supernova shell in which physical conditions are not yet perfectly understood. Third, more photoelectric observations of supernovae are required to support the assumption that all SNI have the same luminosity at maximum light. And, fourth, it is not yet entirely certain that SNI at maximum light are well represented by spherical black bodies

with a temperature of 20,000 K.

As long as such uncertainties remain, the search will continue for additional calibration techniques for the extra-galactic distance scale. New urgency has been given to this search by the recent work of A.R. Walker (*Mon. Not. R. astr. Soc.* 212, 343; 1985) which suggests that the calibration of the Cepheid distance scale may be in error by as much as 0.2–0.3 mag. Promising new methods for the calibration of the extra-galactic distance scale, which have not yet been fully exploited, involve observation of the light curves of distant novae (two of which have recently been discovered in the Virgo cluster galaxy M87) and comparison of the luminosity functions of distant globular systems with those of M31 and the Galaxy. Such determinations are now possible by detecting the maxima of the luminosity functions for the distant globular cluster systems surrounding NGC3379 and M87. The ultimate solution to the distance-scale problem may have to await the Space Telescope. □

Sidney van der Bergh is at the Dominion Astrophysical Observatory, Victoria, British Columbia V8X 4M6, Canada.

Palaeontology

Scottish window on terrestrial life in the Lower Carboniferous

from Andrew R. Milner

ONE of the areas of the world that has the greatest potential for filling some of the gaps in our knowledge of the land fauna before the middle stage of the Upper Carboniferous — the Westphalian (316–296 Myr) — is the Scottish Midland Valley around and between Edinburgh and Glasgow. This area is covered by sequences of freshwater, estuarine and marine beds and there are many coal-mines and surface workings cutting through the appropriate horizons. Over the last decade, the palaeontological community has benefited greatly from sustained prospecting in that area by Mr Stanley Wood. Amongst other new sites, he has revealed an early Upper Carboniferous lake-bed assemblage of aquatic amphibians and fishes at Cowdenbeath^{1,2}; an early Upper Carboniferous coastal assemblage of fishes and crustaceans at Bearsden³; and, as reported on page 355 of this issue, the first Lower Carboniferous assemblage of terrestrial amphibians, arthropods and plants at East Kirkton⁴.

The origin and early diversification of terrestrial animal life is a vast palaeontological and evolutionary jigsaw of which we still have only a handful of pieces. The first certain terrestrial arthropods — from the Rhynie Chert and the Old Red Sandstone — are of Lower Devonian age (410–400 Myr), whilst the earliest known amphibians are Upper Devonian (around

360 Myr) and already show few relictual fish-like characteristics. To cover the intervening time span, some half-dozen localities have produced only fragments of information about terrestrial arthropods, although the recently reported Middle Devonian locality at Gilboa, New York State⁵ shows great promise. The record has until now been little better for the early Carboniferous and it is not until the Westphalian that palaeobiologists can study comprehensive assemblages of myriapods, arachnids, insects, molluscs and vertebrates. Many groups of terrestrial animals make their first appearance as fossils in the Upper Carboniferous, in particular the winged insects and reptiles, but their very structural diversity at this time speaks for a long previous history. By the time we can see what is happening on land, many of the most interesting events have already taken place.

This, then is the significance of the East Kirkton assemblage, which is quite unlike any previously described association of Lower Carboniferous/Mississippian animals. It seems to be in a spherulite-bearing freshwater limestone laid down in shallow pools associated with hot springs⁶. No fish or benthic invertebrates are present, suggesting that the pools were generally uninhabitable. To my knowledge, only two Palaeozoic sites, both younger, show environmental similarity

to that at East Kirkton. One is an algal limestone from Hamilton in Kansas, which appears to be a very similar matrix but without the spherulites, and is of late Pennsylvanian or early Permian age. It has produced a similar association of temnospondyls, arthropods and plants⁷ but differs in the presence of at least one genus of fish being present in abundance⁸. A Lower Permian freshwater limestone of rather different character from Niederhässlich, near Dresden, also has a large fauna of small amphibians and reptiles without fish⁹. A comparative study of these sites might ultimately permit us to identify common geological factors and so to predict further such localities.

The predominant amphibian in the East Kirkton assemblage bears a close resemblance to *Dendrerpeton*, a temnospondyl amphibian from the early Westphalian of Nova Scotia and Ireland^{10,11}. The Nova Scotia material was found in accumulations inside hollow lycopod stumps, suggesting preservation under terrestrial conditions¹², and it is generally believed that *Dendrerpeton* was a terrestrial animal. The East Kirkton amphibian is the earliest certain temnospondyl, showing the definitive large tympanic notch and the huge openings in the palate that characterize the amphibian order Temnospondyli and its descendants — the frogs.

The few months of collecting at East Kirkton have produced intact amphibians, millipedes, eurypterids, scorpions, the earliest harvestman and much plant material, some of it three-dimensionally preserved in chert. If collecting can be extended, the material should establish whether the Euramerican Lower Carboniferous continental fauna was significantly more primitive than its Upper Carboniferous equivalent. If they were similar, the problems of early terrestrial evolution and diversification are pushed back into the Devonian.

As one of the earliest sites to produce most of the components of a terrestrial ecosystem, East Kirkton deserves comprehensive investigation. But there are difficulties in attracting funds for such projects, which

makes it increasingly necessary for palaeontologists to adopt a higher public profile. Following the successful dinosaur exhibition put on by York Museum with the cooperation of the British Museum (Natural History), the Hunterian Museum of the University of Glasgow is preparing an exhibition based on Stanley Wood's remarkable collections, to include some of the East Kirkton material and the spectacular Bearsden fishes. It will travel around museums in Scotland and visit the British Museum (Natural History) in London during 1986. □

Cytoskeleton

Myosin filaments in cytoplasm

from Thomas D. Pollard

EVERY so often a quiet and contented field of research is awakened by a technical advance that at once changes the conventional wisdom and opens up new avenues for experiments. The paper by Yumura and Fukui in the 14 March issue of *Nature*¹ should have this impact on investigation of myosin filaments in non-muscle cells.

Myosin is best known as a filamentous protein of muscle, in which, together with actin, it plays a major role in the process of contraction by the sliding-filament mechanism. Much less is known of the function of myosin in non-muscle cells and its presence in filamentous form has been in doubt. Yumura and Fukui have shown for the first time, in either the light or electron microscope, both the extent of polymerization of myosin and the distribution of its filaments inside a non-muscle cell. Yumura and Fukui devised a simple method to visualize the filaments. The trick involves flattening live cells with an agar overlay¹ to create a more or less two-dimensional cell, in which fine details are not obscured by superimposition, and individual myosin filaments, stained with a fluorescent antibody, can be resolved in the light microscope². Although only demonstrated for the cellular slime mould *Dictyostelium discoideum*, the technique should be widely applicable.

Previous work with fluorescent antibody staining of vertebrate cells in culture has established that myosin is associated with actin-containing stress fibres^{3,4} and the cleavage furrow during cell division⁴. The staining was often reported to be punctate, but it has not been possible to resolve individual filaments. It has also been difficult convincingly to demonstrate myosin filaments in non-muscle cells by electron microscopy, even using antibodies⁵⁻⁷. The general view has been that myosin filaments are present, but too small to visualize by light microscopy and so few in number that they are lost to view in electron micrographs among the abundant actin filaments.

The new technique makes it possible to identify individual rod-shaped particles in

1. Andrews, S.M. *et al. Nature* **265**, 529 (1977).
2. Smithson, T.R. *Palaeontology* **23**, 915 (1980).
3. Wood, S.P. *Nature* **297**, 574 (1982).
4. Wood, S.P., Panchen, A.L. & Smithson, T.R. *Nature* **314**, 355 (1985).
5. Shear, W.A. *et al. Science* **224**, 492 (1984).
6. Muir, R.O. & Walton, E.K. *Trans. Geol. Soc. Glasg.* **22**, 157 (1957).
7. Mapes, G. & Rothwell, G.W. *Palaeontology* **27**, 69 (1984).
8. Zidek, J. *Paleont. Contr. Univ. Kansas* **83**, 1 (1976).
9. Credner, H. *Naturwiss. Woch.* **5**, 471 (1890).
10. Carroll, R.L. *J. Paleont.* **41**, 111 (1967).
11. Milner, A.R. *Palaeontology* **23**, 125 (1980).
12. Carroll, R.L. *et al. Field Excursion A59 Guidebook*, 24th Int. Geol. Congr., Montreal (1972).

Andrew R. Milner is in the Department of Zoology, Birkbeck College, Malet Street, London WC1E 7HX, UK.

cells that are stained with a fluorescent antibody to myosin. Although there is no direct proof that these particles are myosin filaments, it is very likely so, because they are the same size and shape as the filaments prepared from purified myosin.

Yumura and Fukui are able to make several important points on the basis of their observations. First, the bulk of the myosin is polymerized into filaments in the cell. Second, they confirm that myosin is concentrated in the cleavage furrow of dividing cells but add that the filaments are oriented parallel to the band of actin filaments that encircles the equator of the cell. This is the alignment expected if the myosin interacts with actin filaments in a sliding-filament mechanism to produce the force required for division. This observation strengthens arguments for such a model^{4,9,10}. Third, they show that cyclic AMP, the chemoattractant for *Dictyostelium*, causes a striking transient redistribution of the myosin filaments in the cell. This change may very well be a primary step in the chemotactic response of the cell to cyclic AMP and we can look forward to learning whether it is caused by phosphorylation of the myosin¹¹ or some other mechanism. Finally, evidence is provided that the myosin filaments are destroyed by treatment with osmium. If this is true for myosin in other cells, it may well explain previous difficulties in demonstrating myosin filaments by electron microscopy □

1. Yumura, S. & Fukui, Y. *Nature* **314**, 194 (1985).
2. Yumura, S., Mori, H. & Fukui, Y. *J. Cell Biol.* **99**, 894 (1984).
3. Weber, K. & Groeschel-Stewart, U. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4561 (1974).
4. Fujiwara, K. & Pollard, T.D. *J. Cell Biol.* **71**, 848 (1976).
5. Herman, I.M. & Pollard, T.D. *J. Cell Biol.* **88**, 346 (1981).
6. Drenckhahn, D. & Groeschel-Stewart, U. *J. Cell Biol.* **86**, 475 (1980).
7. Hirokawa, N., Tilney, L.G., Fujiwara, K. & Heuser, J.E. *J. Cell Biol.* **94**, 425 (1982).
8. Clarke, M. & Spudich, J.A. *J. molec. Biol.* **86**, 209 (1974).
9. Schroeder, T.E. *J. Cell Biol.* **53**, 419 (1972).
10. Mabuchi, I. & Okuno, M. *J. Cell Biol.* **74**, 251 (1977).
11. Kuzmarski, E.D. & Spudich, J.A. *Proc. natn. Acad. Sci. U.S.A.* **77**, 7292 (1980).

Thomas D. Pollard is in the Department of Cell Biology and Anatomy, John Hopkins Medical School, Baltimore, Maryland 21205, USA.



Scorpion ($\times 4$) from East Kirkton.

Plasma physics

Fusion in magnetized plasmas

from A.C. Riviere

FUSION of light nuclei under controlled conditions promises to be a source of energy for electricity generation in the future. The latest achievements in plasma physics and controlled nuclear fusion, many of which were reported at a recent conference*, make it clear that considerable progress has been made towards the conditions needed for 'ignition', when energy lost from the plasma is matched by energy gained from alpha particles produced by fusion reactions. In a plasma that is half deuterium and half tritium, this requires the product of nuclear density (N) and energy confinement time τ_E to exceed about $2 \times 10^{20} \text{ m}^{-3} \text{ s}$ with the temperature above 10 KeV (10^8 K).

The main approach to fusion has been to use a magnetic field to isolate the hot plasma from the vacuum vessel wall, and the most successful magnetic field geometry is that of the 'tokamak' which combines a strong toroidal field with the field due to a toroidal current flowing in the plasma. A record value of $0.8 \times 10^{20} \text{ m}^{-3} \text{ s}$ for $N\tau_E$ at 1.5 KeV has been achieved in the Alcator C tokamak at MIT (M. Greenwald) by injecting solid hydrogen pellets into the ohmically heated plasma. This method of refuelling raises N without causing a reduction in τ_E . In a much larger tokamak, Doublet III at San Diego, a value of $0.14 \times 10^{20} \text{ m}^{-3} \text{ s}$ for $N\tau_E$ at 6 KeV has been achieved by combined neutral-beam heating and hydrogen-pellet refuelling in a divertor configuration. In the latter variant of the tokamak, magnetic field lines are arranged to connect the plasma edge to a remote collector rather than have the plasma edge defined by a local solid surface. This allows some control over the

flow of impurity ions into the plasma and thereby a reduction in radiation losses. Experiments at ASDEX, Max Planck Institut, Garching and PDX, Princeton, also show improved performance with a divertor, particularly in allowing the maintenance of a second plasma state, called the H-mode, the τ_E of which is about a factor of 2 better than that of the more usual state. In the H-mode a steep rise in temperature occurs at the plasma edge but whether the divertor configuration is essential for this to be formed is not yet clear.

Although plasma densities and temperatures close to those required for ignition have been achieved separately at various times, it remains difficult to reach the necessary energy confinement time. Energy is lost from the plasmas in fusion experiments at an anomalously high rate compared with that expected from the effect of electron-ion collisions. Until recently the scaling of τ_E with size for tokamaks was thought to vary as the square of the scale size — in particular, $\tau_E \propto a^2$, where a is the minor radius of the plasma — so larger and larger machines have been built. With results now available from a wide range of machines it is, instead, found that τ_E increases as the cube of the scale size, probably as $\tau_E \propto aR^2$, where R is the major radius of the torus (P.C. Efthimion, TFTR, Princeton). The Joint European Torus (JET), the largest tokamak built so far with $R = 2.96 \text{ m}$ and $a = 1.25 \text{ m}$, started operating about a year ago and its performance is at least as good as was originally hoped — with τ_E about 0.8 s — and confirms the scaling with aR^2 (P. Rebut and J.G. Cordey). Plasma currents approaching 4 MA have been achieved in JET and 25 MW of additional plasma heating power is soon to be installed. This is expected to drive the plasma density and

temperature up to the conditions where the power deposited by the α -particles from thermonuclear reactions will play a major role (P. Rebut).

Many new experiments have been successful in improving energy confinement. For example, injection of deuterium instead of hydrogen beams into deuterium plasmas at Doublet III and ASDEX, refuelling with pellets rather than feeding gas at the plasma boundary at Alcator C and Doublet III, addition of low Z impurity ions at ISX-B, Oak Ridge, and the use of magnetic divertors at ASDEX, Doublet III and PDX. An explanation for all these effects did not emerge at the meeting, although careful construction of the relative shape of temperature and density profiles may be one factor in minimizing the cross-field energy flow to the walls.

A clear answer is, however, emerging on the maximum value of the ratio (β) of plasma to magnetic-field pressure that can be supported by tokamaks with present techniques. Many experiments have found a β limit, independent of the form of heating, given by $\beta = \text{const} \times I/(aB)\%$, where I is the plasma current and B the value of the toroidal field at the plasma centre. The maximum value of the constant achieved in experiments at Doublet III, ASDEX, PDX, and both CLEO and TOSCA (Culham), is about 3.5, which is in quite good agreement with theoretical calculations by several groups. Beyond this value of β , the τ_E decreases rapidly or the plasma completely breaks up ('disrupts') and the plasma energy is lost. The currently observed limit to β is rather restrictive for present thermonuclear reactor designs based on the tokamak, because their values of I/aB are too small. Some re-thinking of typical reactor parameters is now needed. It should be recalled that there is a limit (the Kruskal-Shafranov limit) above which the plasma current of a tokamak cannot be increased; at higher currents the plasma usually disrupts and terminates the discharge.

The ability to contain a higher value of β is expected for the reversed field pinch (RFP) — a toroidal magnetic system in which the toroidal field is reduced relative to that of a tokamak and is comparable with the poloidal field. An RFP achieves gross stability by reversing the toroidal field in the outer plasma layers, thereby providing a region where the field-line pitch changes rapidly with radius (high shear). Although the development of RFPs is somewhat behind that of the tokamak, D.A. Baker (Los Alamos National Laboratory) reported that an electron temperature of 0.5 KeV has been achieved in the ZT-40M experiment with a plasma current of 0.4 MA; the discharge duration had been improved from a few ms to 27 ms at lower currents. Similar progress was described by P.G. Carolan (Culham) from the performance of the HBTX1A experiment. These parameters move the RFP performance towards that of tokamaks of the

*10th international conference on 'Plasma Physics and Controlled Nuclear Fusion', Imperial College, London 12-19 September 1984.



100 years ago

THE PEABODY MUSEUM AT NEW HAVEN, U.S.

THE Peabody Museum, Mr. Ingersoll informs us, stands on the corner of Elm and High Street, just without the campus of Yale College. The building is due to the liberality of George Peabody, who gave a sum of money, in 1866, to erect a house for the collections. Thanks to the financial prosperity of Massachusetts, the bonds for a hundred and fifty thousand dollars had greatly increased, and those set aside for the first wing of the building had become worth a

hundred and seventy-five thousands dollars when the trustees began to build. With that sum they have erected one of the finest buildings, for its purpose, in the United States—a lofty and ornamental structure of red brick and cream-coloured stone, whose broad and numerous windows express the desire of the investigators within for all the light they can get.

The glory of the Yale Museum is its palaeontological treasures, brought together wholly by Prof. O. C. Marsh. The few representatives of this collection visible in the second-floor and in the hall-ways are alone sufficient to stamp the museum as pre-eminent of this line; but they are merely an advertisement of what cellar and attic contain. It is not too much to say that, in respect to vertebrate palaeontology (outside of fishes), this museum is not surpassed in the world. Where other collections own fragments or single skeletons, Prof. Marsh boasts scores or hundreds of individuals.

From *Nature* 31 510, 2 April 1885.

same size and, according to one theoretical model, the performance of an RFP at the reactor level could exceed that of the tokamak (J.B. Taylor, Culham).

Another approach to maintaining a stable discharge at high values of plasma current is to apply a helical field of the correct helicity from outside. OHTE was built to test this hypothesis and T. Tamano (San Diego) reported that a plasma current of 0.5 MA had been maintained in OHTE for several milliseconds, giving electron temperatures of 0.5 KeV, although the desired field configuration may not have been completely established. The β values achieved by the RFPs and OHTE are around five per cent and so comparable with those from well-maintained tokamaks, but are obtained at the cost of very high power input, indicating a very short τ_E . Thus, to make their use in power stations more economically attractive, tokamaks will need higher β values, whereas the RFP and OHTE devices will need to achieve very much larger values of τ_E .

The spheromak type of configuration has affinity with the RFP but the plasma is formed as a free toroid that generates its own field so that a central conductor through the hole in the plasma doughnut is not required. M. Yamada (Princeton) reported successful generation and control of such plasmas in the S1 spheromak for a period of one millisecond at a plasma current of 0.35 MA, but with an electron temperature of only ~ 50 eV. The CTX (Los Alamos) and CCTC-I (Osaka) experiments have also generated such plasmas. Although at an early stage of development, these intriguing devices should tell us a lot about the ability of magnetized plasmas to regulate themselves by automatically relaxing and maintaining a preferred magnetic geometry.

Stellarators are an important class of field configurations which require no plasma current and entirely confine the plasma by the use of externally-generated helical fields. Heliotron E is stellarator with a highly-sheared magnetic field. It can achieve a β value of 2 per cent (K.Uo, Kyoto) and more power may take the value yet higher. Theoretical limits to β in stellarators, discussed by B.A. Carreras (Oak Ridge) and J. Johnson (Princeton), are comparable to those for the tokamak. Wendelstein VII A (Garching) is a weak shear $\lambda=2$ stellarator in which, like Heliotron E, the plasma is heated both with neutral beams and with absorption of microwaves at the electron cyclotron resonance. In this machine, τ_E shows a strong sensitivity to the rotational transform with large dips in τ_E at rational values (H. Renner, Garching). The weak shear in this case probably allows large regions of the plasma to be interconnected because of the effect of small resonant perturbations. Overall, however, stellarators are now improving on the performance of tokamaks of comparable size, and producing τ_E values closer to the classical values

expected due to collisions alone. Their value of τ_E can therefore be expected to increase as B^2 , a feature not observed in tokamaks; this should be confirmed in the new devices now being constructed at Garching (WVIIAS) and at Oak Ridge (ATF). As stellarators do not require a plasma current they can be operated continuously without plasma disruption. The complex coil system required to establish the helical field is a serious drawback to stellarators but simpler coil designs, which would avoid most of the engineering problems, have been proposed.

The mirror machines, with their essentially linear geometry, are the most studied non-toroidal systems. Many of these devices, some quite large, are in operation. Very ingenious techniques are used to prevent electrons, ions and energy being lost too rapidly along the field lines. In TMX-U (Livermore) and Gamma 10 (Tsukuba), the formation of potential barriers and improved energy confinement have been demonstrated. However the plasma parameters in the centre ($N = 6 \times 10^{18} \text{ m}^{-3}$, $T = 0.2 \text{ KeV}$ and $\tau_E = 5 \text{ ms}$) are still modest compared with toroidal systems.

The heating of plasmas above that provided by the plasma current is technically difficult and has, until recently, relied on injection of fast particle beams. Heating by the absorption of radio-frequency waves has now advanced to the point where MWs of power can be delivered to the plasma. In particular, at the electron cyclotron resonance, radiation with wavelengths of 3.6 and 5 mm and a power level of 1 MW has been used very effectively both in the Doublet III tokamak (K.H. Burrell) and in the T10 tokamak in Moscow (V.V. Alikae). The local nature of the power deposition with this form of heating has been used to study energy transport and instability control.

There is no doubt that exciting progress in fusion research has been made in many directions, although extensive development work will be needed for economic power generation from fusion. The next significant step in fusion research is going to be the achievement of ignition and many now believe this can be done. $\square$

A.C. Riviere is at the Culham Laboratory, Abingdon, Oxon OX14 3DB, UK.

New constraint on biomedical research?

THE recent prosecution of the Royal College of Surgeons, Parliamentary moves to replace the Cruelty to Animals Act (1876) and the search for *in vitro* alternatives to animal experimentation may have far-reaching effects on research in Britain. A further step in this often acrimonious debate was taken at the inaugural meeting of a new pressure group in Oxford last week.

The conference was opened by the ecologist, Dr J.H.C. Finch. He pointed out that we are, of necessity, closely linked with all the living organisms with which we share the planet, and that we ignore this at our peril. The wholesale destruction of ecosystems, nuclear proliferation and unethical experimentation were all part of the same anti-life attitude.

Citing electrophysiological evidence that even plants, although they lack a demonstrable nervous system, are capable of responding to painful stimuli, Dr Finch said, "We should respect and revere all the organic world". In the following discussion, several participants pointed out that large and effective organizations already exist for animal rights and suggested the formation of a parallel group to protect plant life. One delegate suggested the term 'chlorophyll based organisms' or CBOs, because they felt the word 'vegetable' can carry derogatory connotations. However, after debate, the name Vegetable Liberation Front was accepted for the new organization.

On the question of agriculture, it was agreed to demand that the government support a major research initiative to develop methods for the *de novo*

molecular synthesis of foodstuffs. Until this was achieved, the spraying of crops with local anaesthetic before reaping was recommended. No agreement was reached on the ethics of genetic engineering of bacteria for mass production of nutritional protein. One faction insisted that transfection is a violation of genetic integrity, and that this constitutes a subtle form of oppression. "Even bacteria have rights", said one speaker.

There was a strong feeling expressed by many that the movement could only develop, like the Animal Liberation Front, through direct action. Tactics for harvest sabotage, ranging from the immobilization of tractors (but not by stuffing potatoes in their exhaust pipes) to the occupation of grain silos were discussed. Attacks on herbicide factories were rejected because "they could serve a useful role in culling the human predator." The international nature of the struggle was stressed by a fraternal delegate from Holland. He proposed simultaneous demonstrations throughout the EEC outside offices of Interflora, a multinational corporation accused of cruelty to flowers.

Given the urgency of the problems raised, the question of a constitution was deferred until a subsequent meeting. However, a manifesto was drawn up and agreed by the overwhelming majority of delegates. The document was engraved on tablets of stone, since the manufacture of paper was held to entail the suffering of trees, and a copy was posted through windows of the editorial offices of this and other journals.

R.A. Tatouille, University of Brussels.

Amazonian Indians and the forest environment

SIR — The statement in a recent *News and Views* article by May¹ that Amazonian Indians possess a much greater knowledge of their environment than has been commonly believed prompts me to communicate some data gathered recently that further support this contention.

The Institute of Economic Botany of The New York Botanical Garden (supported by E. J. Noble Foundation) is engaged in a four-year comparative ethnobotanical study on the question "How important is the forest to Amazonian Indians?" Much has been written on the significance of the forest to Amazonian Indians², but rarely has the degree of importance been quantified, and then only on a very small scale³. A primary goal of the project is to quantify forest utilization for 1 hectare of forest with each tribe studied.

The Chacobo Indians of Bolivia were the first to be studied⁴. Just 30 years ago, the tribe numbered only about 135 individuals who essentially lived outside the Bolivian cash economy by hunting, fishing, and subsistence agriculture in isolated forest locations along the Ríos Ivon, Benicito, Geneshuaya and Yata in the northeastern Bolivian department of Beni⁵⁻⁷. In 1955, modern missionary work with the Chacobo began and in the ensuing years a number of aspects of their culture, including their ceremonies, traditional mode of dress, and supernatural beliefs have been lost. Other aspects, including their botanical knowledge, are to a greater degree intact, at least among the elders. Although their numbers have increased to about 400, their culture is on the verge of extinction. The majority of the tribe (some 280 individuals) live today on 43,000 hectares of forested land along the Río Ivon, and on this reserve make their capital village, Alto Ivon (11°45'S, 66°02'W). The vegetation around Alto Ivon is Tropical Moist, terra firme forest. The elevation is about 200 m and the topography is level. The inventory, which was carried out in January and February 1984, was made on a strip of undisturbed forest 10 × 1,000 m. Every tree with a DBH (diameter at breast height) of 10 cm or more was marked and collected. Indigenous names and use information were obtained from Chacoba informants.

The inventory showed that 91 species, represented by 649 individual trees, occurred in the hectare. Of these, the Chacobo had uses for 75 species (82% of the total), or 619 individual trees (95% of the total). The species were grouped into five classes of utilization: food, fuel, crafts and construction, medicinal, and commercial. Some species, especially the palms, had uses in more than one category. There was only one commercially exploited species, wild rubber (*Hevea brasiliensis*), and 5 individuals of that species were found. Fourteen species (163 individuals) were used specifically as a fuel for cooking, smoking

rubber, or both. Thirty-three species of trees (264 individuals) provided edible wild fruits. Twenty-three species (225 individuals) were collected that were used in crafts and construction. Finally, 23 species of trees (271 individuals) were found in the sample hectare that were used by the Chacobo for medicinal purposes.

The demonstration that the Chacoba utilize 82% of the tree species in their environment has significant implications not only for the thesis that such tribes have a profound knowledge of their forest, but also for those individuals concerned with the protection of indigenous cultures in Amazonia. For example, by combining this sort of ethnoecological information with the data being obtained from the Minimal Critical Size of Ecosystems Project (co-sponsored by the Brazilian National Institute for Amazon Research and the World Wildlife Fund-US)⁸, it should be possible to make more intelligent recommendations as to how much forested land must be allocated for self-sustaining Indian reserves.

Every ethnobotanical study done in Amazonia reaffirms the view that indigenous peoples are scientifically interesting, legitimate and invaluable sources of knowledge about the forest. Since one tribe differs from another in important ways, and since these people are so rapidly losing their traditional knowledge, many more studies are urgently needed. It is clear that botanical inventory involving indigenous cultures, especially in the tropics, can quite justifiably be considered the most worthwhile expenditure of time and effort for this generation of field botanists.

BRIAN M. BOOM

*The New York Botanical Garden,
Bronx,
New York 10458, USA*

1. May, R.M. *Nature* 312, 19-20 (1984).
2. Lévi-Strauss, C. in *Handbook of South American Indians* Vol.3, (ed. J.H. Steward) 465-486 (Smithsonian, Washington, 1950).
3. Carneiro, R.L. in *The Nature and Status of Ethnobotany* (ed. R.I. Ford) 201-216 (Univ. of Michigan, Ann Arbor, 1978).
4. Webster, B. *New York Times*, Sunday, November 4, 1984.
5. Métraux, A. in *Handbook of South American Indians* Vol.3, (ed. J.H. Steward) 449-452 (Smithsonian, Washington, 1948).
6. Prost, M.D. *Costumbres, Habilidades y Cuadro de la Vida Humana Entre los Chacobos* (Instituto Lingüístico de Verano, Riberalta, Bolivia, 1970).
7. Torricco, P.B. *Indígenas en el Corazón de América* (Los Amigos del Libro, La Paz, Bolivia, 1971).
8. Lewin, R. *Science* 225, 611-612 (1984).

Electrical aspects of the snowflake crystal

SIR — The discussion "No pattern yet for snowflakes"¹ laments the inability of Vicesek's calculations² to reproduce the long-range symmetry observed in snowflakes. In the closing lines it is suggested that adding lattice vibrations to the calculations may be helpful. Because snowflakes are polycrystalline conglomerates there would seem to be little chance that these very short wavelength vibrations could be the mediating phenomenon for symmetry patterns that

are many millimetres in size. Long wavelength vibrations have been proposed by McLachlan³ and later by Tolansky⁴. An inherent drawback in any vibrational theory is that the details of the observed structure must be matched by a spectrum of vibrations with equivalent complexity in amplitude, frequency, and phase. In addition, a mechanism for the generation, transmission, and maintenance of these vibrations must be found.

A simpler, more direct mechanism is desirable to explain the generation of the observed cooperative behaviour in dendrite growth. I have proposed a mechanism that will provide the long-range symmetry observed based on phenomena known to be present during the snowflake development⁵. The fact that snowflakes carry an electric charge has been observed by many workers in the field. The influence of electrical fields on ice crystal growth has been observed by Zawadzki and Papée⁶, as well as by Muchnik and Rud'ko in the Soviet Union. The mechanism of symmetry induction is the accretion of water vapour at those sites on the crystal that lead to the lowest potential-energy state. There are twelve symmetry axes to the common snowflake. The axis of each dendrite and the bisector of the angle between dendrites serve as reflection planes to sequentially propagate the pattern to all dendrites. Pohl has described the behaviour of dielectric materials in divergent fields and its commercial application⁷.

It is interesting to note that this symmetry-inducing mechanism operates regardless of the sign of the charge of the snowflake, which may change during the growth period, or whether the snowflake is increasing or diminishing in size. It is also independent of the number of dendrites, or their length or configuration.

An examination of a collection of photographs of snowflakes, such as that of Nakaya⁸, leaves one impressed by the varieties of type and detail of the symmetry achieved in snowflakes.

It would be most interesting if future calculational models of snowflake growth would include the influence of their external electrical field. Crystal growth from the liquid phase will not show phenomena dependent on an external electrical field and thus cannot duplicate the symmetry found in snowflakes. It is interesting to note that this restriction would not necessarily apply if vibrational processes were the source of symmetry.

ROALD A. SCHRACK

*National Bureau of Standards,
Gaithersburg,
Maryland 20899, USA*

1. Maddox, J. *Nature* 313, 93 (1985).
2. Vicesek, T. *Phys. Rev. Lett.* 53, 2281 (1984).
3. McLachlan, *Proc. natn. Acad. Sci. U.S.A.* 43, 143 (1957).
4. Tolansky *Nature* 181, 256 (1958).
5. *J. Wash. Acad. Sci.* 48, 273 (1958).
6. Zawadzki & Papée *Nature* 196, 568 (1962).
7. Pohl *J. appl. Phys.* 22, 869 (1951); 29, 1182 (1958).
8. Nakaya, U. *Snow Crystals, Natural and Artificial* (Harvard University Press, 1954).

Source of Precambrian chemical and clastic sediments

R. G. Miller & R. K. O'Nions

Department of Earth Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EQ, UK

The Nd isotope composition of chemical and clastic sediments surviving from the Archaean provides no evidence for the existence of pre-4,000-Myr continental crust. Either the amounts of such crust were trivial or it was destroyed substantially by 3,800 Myr ago. Nd in major Precambrian banded iron formations was supplied largely by continental weathering, and in this respect the water masses in which they formed were analogous to the modern oceans.

THE oldest surviving vestiges of undisputed continental crust are no older than 3,800 Myr. If a substantial amount of continental crust existed before then, it must have been destroyed and essentially lost from the record. Materials eroded from such an early crust, however, might have contributed to later Archaean sedimentary systems.

Clastic and chemical sediments deposited throughout the Phanerozoic differ from those deposited earlier in Earth history in several important respects. One of the most pronounced differences concerns the apparent absence of banded iron formation (BIF) in the Phanerozoic and its abundance in the Archaean and early Proterozoic¹⁻³. In the present-day weathering environment, Fe(II) is readily oxidized to Fe(III), which, because of its low solubility in normal pH ranges, suffers little transport. As a consequence, the concentration of Fe in sea water is far too low ($\sim 3 \times 10^{-6} \text{ g l}^{-1}$)⁴ to produce extensive Fe-rich sediments, such as those once deposited in the Hamersley Basin ($\sim 2 \times 10^{10} \text{ kg Fe annually}$)⁵. At the time of BIF deposition, substantially higher concentrations of Fe may have developed in sea water, possibly in response to more reducing conditions in the oceans⁶.

Important changes may have occurred also in the clastic sedimentary record. For example, Taylor and colleagues⁷⁻⁹ have shown that many Archaean sediments are characterized by relatively low abundances of Th and U and the absence of a Eu anomaly, whereas Proterozoic fine-grained clastic sediments possess both higher Th and U abundances and a pronounced Eu anomaly. These differences have been interpreted to reflect a fundamental change in the character of the exposed continental crust. It remains to be demonstrated, however, whether or not the Archaean clastic sediments that have survived to the present (now found largely in greenstone belts) are representative of the entire sedimentary mass as it existed at those times. If the inferred changes in the chemical character of the continents are indeed real, and not an artefact of selective sampling or sediment preservation, then their implications for the changing nature of continental differentiation are profound.

The question of the provenance of Precambrian chemical and clastic sediments and their implications for the development of the exposed continental crust are investigated here by the measurement of ¹⁴³Nd abundances in Precambrian sediments. The use of the ¹⁴³Nd natural radiogenic tracer (α -decay product of ¹⁴⁷Sm; $t_{1/2} = 1.06 \times 10^{11} \text{ yr}$) derives from the $\sim 40\%$ lower Sm/Nd ratio of the continents compared with the mantle. The uniform Sm/Nd ratio of fine-grained clastic sediments, together with a complementary evolution of ¹⁴³Nd in the continents and the mantle, renders sediments from old and juvenile crustal sources readily distinguishable, even when these sources have been reworked and recycled in the crust¹⁰⁻¹⁶. The relationship allows an estimate to be made of the average residence time of the Sm and Nd components in the continents rather than the mantle ($= t_{\text{CR}_3}$, or model crustal residence age)¹². It is well established¹⁷⁻²⁰ that the isotope composition of modern seawater Nd reflects a major input from continental sources and

that this signature is acquired by chemical sediments presently forming in the ocean basins. On this basis, Precambrian BIFs are expected to acquire their Nd from the water bodies from which they were precipitated and thereby provide information on the nature of the sources involved.

Sm-Nd isotope data are reported here for Archaean fine-grained clastic sediments from Canada and South Africa and for samples of some classic banded iron formations in Africa, Australia, North America and Greenland. Samples from a late Proterozoic (~ 450 –800-Myr-old) BIF in Brazil have also been analysed because they seem to have all the essential features of the older and better-known examples.

Sample selection and techniques

Fine-grained clastic sediments either temporally or spatially associated with BIF occurrences have been selected both to compare the provenance of coeval chemical and clastic sediments and, together with published data, to investigate the sources of Archaean sediments in general (Tables 1, 2). Specifically included are samples from the $\sim 3,450$ -Myr-old Fig Tree and Moodies Groups of the Swaziland Supergroup²¹⁻²⁵, the somewhat younger Pongola Supergroup ($\sim 2,950$ Myr) located south of the Swaziland Supergroup^{21,25,26} and the $\sim 2,700$ -Myr Yellowknife Supergroup of the Canadian Northwest Territories²⁷⁻³⁰.

Samples of oxide ($\pm$ silicate, carbonate) facies banded iron formation have been selected from a number of classic BIFs deposited in the interval $\sim 3,800$ –600 Myr (Table 3). The oldest samples are from the highly deformed and metamorphosed Isua supracrustal belt in south-west Greenland, formed $\sim 3,750$ Myr ago³¹⁻³⁸. Typically, the iron formation at Isua consists of quartz-magnetite-amphibole-carbonate assemblages³⁸⁻⁴⁰. Other samples have been selected from the Animikie Basin of Lake Superior (the Biwabik and Negaunee BIFs, deposited $\sim 2,000$ -Myr ago⁴¹⁻⁴⁴), the Australian Hamersley Basin ($\sim 2,400$ Myr)⁴⁵⁻⁴⁷ and the Fig Tree/Moodies Groups^{21,48} of the southern African Swaziland Supergroup ($\sim 3,450$ Myr)^{21,49-51}. Samples have also been included from the much younger (800–500 Myr) and less well-known BIF in the Jacadigo Series of Morro do Urucum, Brazil^{2,52}. Although this deposit is about an order of magnitude smaller than, for example, the Lake Superior BIF, it seems to be typical of banded iron formations in terms of its mode of occurrence, lithological associations and mineralogy.

Samples of banded iron formation were either sawn into Fe- and quartz-rich bands and analysed separately, or otherwise treated as whole-rock samples in the normal way. All clastic sediment samples were ignited in air to oxidize organic matter. Sample digestion was carried out by conventional techniques using Teflon dissolution vessels. Sm and Nd were separated using a combination of cation-exchange and reverse-phase chromatography as described elsewhere (A. S. Cohen, R.K.O'N. and W. L. Griffin, manuscript in preparation) and mass

spectrometric techniques of isotope analysis followed those described previously^{13,53}. The t_{CR} values in Tables 1 and 2 have been calculated only for those samples with $^{147}\text{Sm}/^{144}\text{Nd} \leq 0.14$.

Results

Clastic sediments. Sm–Nd isotope results for 10 samples of fine-grained clastic sediment collected from greenstone belt associations are presented in Fig. 1 and Table 1. The small range of Sm/Nd ratios ($0.101 < ^{147}\text{Sm}/^{144}\text{Nd} < 0.125$) is comparable with reported ranges for other Archaean (Table 2) and post-Archaean fine-grained clastic sediments and the particulate

loads of modern rivers¹¹. Taken together, these results do not show any significant changes in the Sm/Nd ratio of sediments from the Archaean through to the present day, and do not provide evidence for overall changes to more or less acid compositions. However, note that metasediments with $^{147}\text{Sm}/^{144}\text{Nd} > 0.2$ have been reported from both the Isua and the Malene supracrustal belts of south-west Greenland⁵⁴, a ratio that is substantially higher than the more normal values reported by Hamilton *et al.*¹³.

The ~3,450-Myr-old Swaziland Supergroup shales have crustal residence ages of 3,500–3,600 Myr for those samples with

Table 1 Sm–Nd isotope analyses of banded iron formations and ancient clastic sediments

Sample	Lithology	Nd(p.p.m.)	Sm(p.p.m.)	$^{147}\text{Sm}/^{144}\text{Nd}$ $\pm 0.1\%$ ($2\sigma_m$)	$^{143}\text{Nd}/^{144}\text{Nd}$ $\pm 2\sigma_m$	t_{CR}^* (Myr)	$\epsilon'_{Nd}^\dagger$
Hamersley Basin: 2,400 ± 100 Myr							
120295	BIF whole-rock powder	4.744	1.275	0.1624	0.512022 ± 28	ND	–1.5
120296	BIF whole-rock powder	0.3228	0.08615	0.1613	0.511881 ± 18	ND	–3.9
120301 A	BIF magnetite fragment	3.258	0.7524	0.1396	0.511816 ± 16	ND	+1.6
120301 B	BIF chert fragment	1.254	0.3414	0.1646	0.512105 ± 18	ND	–0.5
103696 A	BIF chert fragment	1.198	0.2694	0.1359	0.511682 ± 18	ND	+0.1
103696 B	BIF chert fragment	0.3674	0.1364	0.2245	0.512841 ± 16	ND	–4.7
103696 C	BIF magnetite fragment	3.700	0.7756	0.1267	0.511491 ± 14	ND	–0.8
103696 D	BIF whole-rock fragment	0.7862	0.2168	0.1667	0.512110 ± 16	ND	–1.1
Fig Tree/Moodies Groups: 3,450 ± 100 Myr							
SL 1	BIF whole-rock powder	1.092	0.3314	0.1835	0.512355 ± 18	ND	+0.3
SL 28 A	BIF whole-rock powder	0.5846	0.1258	0.1301	0.511179 ± 16	3,570	+1.1
SL 28 B	BIF magnetite fragment	0.5138	0.1198	0.1409	0.510955 ± 18	ND	–8.1
SL 583	Fe-shale	3.434	0.7597	0.1337	0.511047 ± 18	3,970	–3.1
SL 9	Shale	4.106	1.200	0.1766	0.512091 ± 14	ND	–1.8
1105	Shale	23.81	4.605	0.1169	0.510863 ± 18	3,570	+0.9
9274	Shale	18.23	3.617	0.1199	0.510975 ± 24	3,510	+1.7
1102	Shale	25.78	4.661	0.1093	0.510694 ± 14	3,560	+0.9
Pongola Supergroup: 2,950 ± 150 Myr							
PM2A	Phyllite	26.40	4.727	0.1082	0.511003 ± 24	3,080	+1.7
PM3A	Phyllite	17.82	3.466	0.1175	0.511320 ± 16	2,890	+4.4
Yellowknife Supergroup: 2,700 ± 100 Myr							
R1	Phyllite	21.79	4.503	0.1249	0.511305 ± 20	3,150	–1.1
R2	Phyllite	28.46	4.748	0.1008	0.511103 ± 22	2,750	+3.4
F63–82	Phyllite	34.13	6.163	0.1091	0.511231 ± 18	2,780	+3.0
Isua Supracrustals: 3,750 ± 50 Myr							
167642	BIF whole-rock powder	1.164	0.3344	0.1736	0.511840 ± 16	ND	–4.5
242573 A	BIF magnetite powder	0.4650	0.1461	0.1898	0.512032 ± 20	ND	–8.6
242573 B	BIF magnetite fragment	1.057	0.2501	0.1430	0.511452 ± 18	ND	+2.9
242573 C	BIF magnetite fragment	0.8122	0.2285	0.1701	0.511806 ± 18	ND	–3.4
242573 D	BIF chert powder	0.5161	0.1300	0.1523	0.511357 ± 18	ND	–3.6
242573 E	BIF chert fragment	0.2654	0.1002	0.2284	0.512658 ± 18	ND	–15.2
242573 F	BIF chert fragment	0.3420	0.1143	0.2020	0.512126 ± 16	ND	–12.7
Animikie Basin: 2,000 ± 100 Myr							
107811	BIF whole-rock powder	34.56	6.884	0.1204	0.511633 ± 14	2,470	0.0
98153	BIF whole-rock powder	7.451	1.103	0.0895	0.511104 ± 16	2,500	–2.4
98154	BIF whole-rock powder	7.674	1.457	0.1148	0.511380 ± 26	2,720	–3.5
98173	BIF whole-rock powder	8.273	1.692	0.1236	0.511676 ± 26	2,490	0.0
Morro do Urucum, Brasil: 650 ± 150 Myr							
URU A	BIF chert fragment	6.455	1.243	0.1164	0.512164 ± 18	1,540	–2.6
URU B	BIF haematite fragment	5.765	1.137	0.1192	0.512162 ± 12	1,590	–2.9
URU C	BIF jasper fragment	21.62	2.795	0.0781	0.512012 ± 16	1,280	–2.4

$^{143}\text{Nd}/^{144}\text{Nd}$ ratios are normalized to $^{146}\text{Nd}/^{144}\text{Nd} = 0.7219$.

* t_{CR} model ages calculated assuming mantle evolution of Nd from $\epsilon_{Nd}^{4,560 \text{ Myr}} = 0$ to $\epsilon_{Nd}^0 = +10$. Thus:

$$t_{CR} = \frac{1}{\lambda} \ln \left(1 + \frac{0.513151 - ^{143}\text{Nd}/^{144}\text{Nd}}{0.2136 - ^{147}\text{Sm}/^{144}\text{Nd}} \right)$$

t_{CR} was calculated only for samples with $^{147}\text{Sm}/^{144}\text{Nd} < 0.14$, for which samples there is a sufficiently large Sm/Nd fractionation relative to depleted-mantle values for the model age to be reliable, assuming a closed-system evolution. ND, not done.

† ϵ'_{Nd} is $10^4 \times$ the deviation of $^{143}\text{Nd}/^{144}\text{Nd}$ measured in a sample from the calculated $^{143}\text{Nd}/^{144}\text{Nd}$ ratio in a CHUR at time t . ϵ'_{Nd} values are calculated for the estimated stratigraphic ages of the samples. Analytical errors result in typical uncertainties in ϵ'_{Nd} of ± 0.6 . The effect of uncertainty in stratigraphic age on ϵ'_{Nd} is illustrated graphically in Fig. 2.

$^{147}\text{Sm}/^{144}\text{Nd}$ ratios <0.14 (Tables 1, 2). Samples SL9 and SL583 yield t_{CR} ages and ϵ_{Nd}^t values (Table 1) inconsistent with the results obtained for the other shales: it may be significant both that the $^{147}\text{Sm}/^{144}\text{Nd}$ ratio found for SL9 is unusually high (0.177) and that SL583 differs from all other samples in containing $>50\%$ Fe_2O_3 . The two phyllites from the Pongola Supergroup have $^{147}\text{Sm}/^{144}\text{Nd}$ ratios typical of fine-grained clastics and t_{CR} values of 2,900 and 3,100 Myr, respectively. For both supergroups, t_{CR} is indistinguishable from the estimated depositional age. Such results indicate that the precursors of the Swaziland and Pongola Supergroup sediments, as for most of the Archaean fine-grained clastic sediments so far analysed (refs 10, 13, 16 and Cambridge unpublished data), had an unresolvable pre-depositional crustal residence and have been derived from essentially contemporary additions to the continental crust presumably associated with the formation of the greenstone belts. However, the normal $^{147}\text{Sm}/^{144}\text{Nd}$ ratio for these sediments indicates that if volcanic additions were a major source, then they must have possessed low Sm/Nd ratios similar to calc-alkaline volcanics. One of the three metasediments from the $\sim 2,700$ -Myr-old Yellowknife Supergroup yields a rather higher t_{CR} age of 3,200 Myr. Although penecontemporaneous volcanics in this greenstone belt may have been major contributors to the sediments, there seems to be a requirement for a contribution from a $\geq 3,000$ -Myr-old source. Whole-rock Rb-Sr ages of 3,100 Myr, and detrital zircons with ages $\leq 3,500$ Myr, have been reported from the Slave craton⁵⁵⁻⁵⁸, and sialic material identified petrologically in the Supergroup sediments²⁷ may be derived from this older basement.

Banded iron formation. Sm-Nd isotope results for 25 samples of BIF are presented in Table 1. These samples include normal powdered whole rocks and, to assess the effects of metamorphism and/or variable provenance of Nd, small samples (≤ 1 g) cut from individual bands. The $^{147}\text{Sm}/^{144}\text{Nd}$ ratios of the BIF reported here are more variable than those of fine-grained clastic sediments. It is well established that fine-grained clastic sediments of all ages exhibit a restricted range of Sm/Nd with $0.09 < ^{147}\text{Sm}/^{144}\text{Nd} < 0.16$ (mean = 0.115)^{10-16,25,54,59,60}, which

contrasts with the larger range for BIF reported in Table 1, where $0.08 < ^{147}\text{Sm}/^{144}\text{Nd} < 0.23$. These results confirm the study of rare-earth elements in BIF made by Fryer⁶¹, which yielded a range $0.09 < ^{147}\text{Sm}/^{144}\text{Nd} < 0.19$. t_{CR} values for BIF have only been calculated for whole-rock samples with $^{147}\text{Sm}/^{144}\text{Nd} < 0.14$, that is, Sm/Nd ratios considerably lower than model mantle values. The concentration of Nd in BIF is usually < 6 p.p.m. (Table 1; ref. 61), but reaches 35 p.p.m. for one of the Lake Superior samples.

In general, the effects of metamorphism on the Sm-Nd isotope systematics of fine-grained clastic sediments are minimal, which renders information concerning their provenance readily accessible. However, the response of the Sm-Nd system in BIF to metamorphism has not been investigated previously, and, if significant, would limit severely the provenance information which can be elicited. The Isua BIF provides an ideal opportunity to assess this possibility because the Isua region was subjected to high-grade metamorphism in the upper amphibolite facies $\sim 3,700$ -Myr ago, and was further metamorphosed at $\sim 2,500$ and $1,600$ Myr^{36,37}.

Specimen 242573 (Table 1) consists of alternating 1–10-mm bands of magnetite or quartz, from which six samples were taken: two are bulk Si-rich and Fe-rich powders respectively, and four are individual adjacent fragments removed from two separate bands. The results obtained (Table 1) are inconsistent with evolution of ^{143}Nd in a reservoir with an initially uniform $^{143}\text{Nd}/^{144}\text{Nd}$ ratio that became a closed system 3,700 Myr ago. $^{147}\text{Sm}/^{144}\text{Nd}$ and $^{143}\text{Nd}/^{144}\text{Nd}$ are correlated positively and indicate mobility of Sm and Nd since 3,700 Myr ago, most probably related to the young metamorphic events identified previously in the Isua area. The perturbation of the Sm-Nd system more than 1,000 Myr after the deposition of the Isua BIF means that the computed initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratios (expressed as ϵ_{Nd}^t values; see Table 1 legend) are not valid estimates for these samples at time t , and suggests that in general ϵ_{Nd}^t values calculated from larger whole-rock BIF samples may be more reliable.

Two whole-rock samples from the $\sim 3,450$ -Myr-old Fig

Table 2 Published Sm-Nd isotope analyses of ancient clastic sediments, $>2,000$ Myr old

Sample	Source	$^{147}\text{Sm}/^{144}\text{Nd}$	$^{143}\text{Nd}/^{144}\text{Nd}$ $\pm 2\sigma_m$	t_{Strat} (Myr)	t_{CR} (Myr)	Refs
Fig tree	Fig Tree Shales	0.1322	0.511193 ± 20	3,350–3,540	3,630	10
KH 44	Australia	0.1220	0.511177 ± 26	2,700–3,000	3,260	10
WC 3	Australia	0.1190	0.510977 ± 24	2,700–2,800	3,470	16
PG 7	Australia	0.1124	0.510609 ± 12	$>3,300$	3,790	16
242579	Isua Supracrustals	0.1026	0.510381 ± 14	3,750	3,770	13
242582	Isua Supracrustals	0.1055	0.510472 ± 14	3,750	3,740	13
242576	Isua Supracrustals	0.1437	0.511403 ± 26	3,750	3,770	13
242565	Malene Supracrustals	0.1103	0.511122 ± 18	2,600–3,000	2,970	13
242562	Malene Supracrustals	0.0945	0.510738 ± 22	2,600–3,000	3,070	13
81062	Baltic Shield	0.1173	0.510987 ± 14	2,800	3,390	Our unpublished data
81064	Baltic Shield	0.1217	0.511301 ± 24	2,800	3,050	

The original grain size of the metamorphosed samples is generally unknown.

* Model ages calculated as for Table 1.

Table 3 Summary geology of sampled banded iron formations

Deposit	Age (Myr)	Mass (kg)*	Thickness (m)	Area (km ²)	Notes	Refs
Isua	3,750	2×10^{12} (ore only)	200 (ore only)	—	Metamorphosed—amphibolite grade. Original size unknown, may be thickened tectonically	31, 35, 38–40
Fig Tree	3,350–3,540	10^{12}	Generally 6–40, <300	—	Low-grade metamorphism. Many small lenses in clastic sedimentary succession	21, 48, 51
Hamersley	2,300–2,500	10^{17}	<350	100,000	~Unmetamorphosed, $T_{\text{max}} \sim 270$ – 310 °C. Five major bands, total 1,000 m thick	45, 47
Lake Superior	2,100–1,850	10^{16}	<240	220,000	Metamorphosed chlorite to sillimanite grades at $\sim 1,860$ Myr	41–43
Urucum	~ 600	10^{15}	>300	$>1,000$	Unmetamorphosed. Contains lenses of manganese oxides, glaciogenic clastics	52

* Mass data from ref. 2.

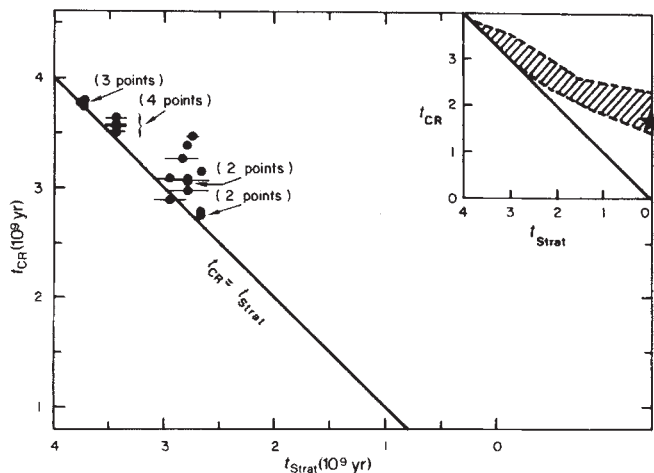


Fig. 1 Comparison of estimated stratigraphic ages (t_{Strat}) and crustal residence ages (t_{CR}) calculated from Sm-Nd isotope analyses (Tables 1, 2). Main graph, Archaean fine-grained clastic sediments. Pre-4,000-Myr contributions to these Archaean sediments cannot be discerned. Most crustal residence ages are ≤ 200 Myr greater than stratigraphic ages. Inset diagram shows that this relationship does not extend into the Phanerozoic; asterisk represents the mean value for fluvial fine-grained present-day clastic sediments (ref. 11); shaded region, world-wide fine-grained clastics.

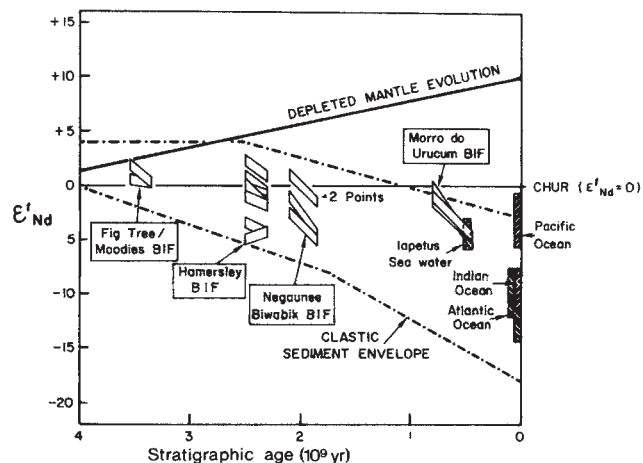


Fig. 2 Banded iron formations. ϵ_{Nd}^t values for samples of BIF, sea water and ferromanganese deposits from modern oceans and metalliferous sediments deposited in Iapetus. ϵ_{Nd}^t values are 10^4 times the deviation of initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratios in a sample from those in a CHUR at time t . Note that all samples have ϵ_{Nd}^t in the range of coeval clastic sediments. The estimated ϵ_{Nd}^t of the depleted mantle excludes a dominant mantle supply of Nd into the majority of BIFs. Isua BIF samples and sample SL28B omitted (see text).

Tree/Moodies Groups BIF, which have been metamorphosed to no more than greenschist facies, yield $+0.3 < \epsilon_{\text{Nd}}^t < +1.1$ (Table 1), although a small fragment from an individual magnetite band yields a very different ϵ_{Nd}^t value of -8 and again suggests small-scale mobility of rare-earth elements. Six single fragments and two whole rocks from the virtually unmetamorphosed Hamersley Basin BIF yield a range of values with $-4.7 < \epsilon_{\text{Nd}}^t < +1.6$. The four whole-rock samples from the Animikie Basin BIF yield a similar range of ϵ_{Nd}^t values ($-3.5 < \epsilon_{\text{Nd}}^t < 0$), and crustal residence ages between 2,500 and 2,700 Myr. The Morro do Urucum BIF is little metamorphosed and yields $-2.9 < \epsilon_{\text{Nd}}^t < -2.4$. The calculated ranges in ϵ_{Nd}^t values for a single BIF are comparable with those in sea water from a present-day ocean basin (Fig. 2), and may reflect real variations in Nd isotope composition in the water masses concerned, or the effects of post-depositional mobilization of Sm-Nd. Although the effects of metamorphic redistribution are severe on the scale of the centimetre banding in the high-grade Isua BIF, they are much less so in the lower-grade BIF. Estimates of the ϵ_{Nd}^t values for the water masses from which Hamersley, Animikie and Urucum BIF were deposited are based consequently on the results from whole-rock samples, but it must be conceded that the small differences between samples could reflect either initial heterogeneities in the water masses and/or post-depositional redistribution of Sm and Nd.

Chemical and clastic sediments

Note that of 18 fine-grained Archaean clastic sediments with $^{147}\text{Sm}/^{144}\text{Nd} < 0.14$ for which reliable crustal residence ages can be calculated, 13 possess t_{CR} and estimated stratigraphic (t_{Strat}) ages that agree within ~ 200 Myr (Fig. 1). Notwithstanding the uncertainty in the correct choice of depleted-mantle models used to calculate the t_{CR} values, this observation supports the notion that the Archaean sediment source was dominated by juvenile material that had not resided as part of the continental crust for more than ~ 200 Myr before its erosion and deposition. No major input of relatively old crust into clastic sediments can be observed until $< 3,000$ Myr ago. Note also that if significant quantities of substantially older crust (that is, $> 4,000$ Myr) existed, then it had either failed to survive by 3,800–3,500 Myr ago, or else sampling to date has not included clastic sediments

derived from this material, and they remain undiscovered. The latter explanation would leave open the possibility that such an ancient source supplied materials to the oceans and might occur in chemical sediments.

The $^{143}\text{Nd}/^{144}\text{Nd}$ ratios measured for modern seawater and pelagic sediments are similar to the global characteristics of the particulate load of the world's major rivers and atmospheric dusts^{11,17–20}. Significant shifts to more positive ϵ_{Nd}^t values in Pacific sea water and ferromanganese deposits may result from a juvenile Nd component with $\epsilon_{\text{Nd}}^0 \approx 10$, such as might be supplied from the oceanic ridges or the relatively youthful continental margins around the Pacific (Fig. 2). Chemical precipitates forming in present-day oceans as manganese nodules or Fe-rich sediments associated with spreading-ridge axes¹⁷ have acquired Nd isotope compositions in the ranges exhibited by the water bodies in which they have formed. It is a reasonable expectation, therefore, that the Precambrian iron formations will have similarly sequestered Nd from the water bodies in which they formed. Hooker *et al.*⁵³ have previously derived estimates for the Nd isotope composition of Iapetus sea water from ~ 490 -Myr-old metalliferous sediments in the Southern Uplands of Scotland. Even though rare-earth mobility may have accompanied burial and diagenesis, it is possible to reconstruct the ϵ_{Nd}^t of the water bodies concerned from Sm-Nd analyses of BIF, as long as rare-earth immobility has prevailed on the scale of sampling from close to the time of sedimentation. It has been demonstrated above, particularly in the case of Isua, that such immobility cannot be presupposed for BIF. However, less metamorphosed BIF and/or larger scales of sampling have yielded results from which details of the provenance of their rare-earth elements can be deduced.

The principal question is whether some special source of Nd, and therefore possibly of Fe, must be invoked to explain the observations on BIF, or whether average crustal sources such as supplied the clastic sedimentary mass during most of crustal history have prevailed. This situation can be assessed using those measurements on BIF thought to yield reliable estimates of ϵ_{Nd}^t , by comparing them with similar observations for the clastic sedimentary mass and for modern and Iapetus ocean water (Fig. 2).

We find that ϵ_{Nd}^t values for the Fig Tree BIF are comparable with those of the contemporary clastic sediments from the Swazi-

land Supergroup, and close to both the chondritic uniform reservoir (CHUR) model or a depleted-mantle model at that time. Thus, the water body from which the Fig Tree BIF was precipitated seems to have received its Nd from a juvenile source whose average previous crustal residence time had not exceeded ~200 Myr. The early Proterozoic BIFs from Lake Superior and Hamersley have variable, mostly negative ϵ_{Nd}^t values, indistinguishable in this respect from their contemporary fine-grained clastic sediments. Similarly, the Morro do Urucum BIF has ϵ_{Nd}^t and t_{CR} estimates similar to those for coeval clastic sediments but which display a bias towards slightly more juvenile Nd, as does modern Pacific sea water. In all the post-Archaean BIFs, the Nd could have been derived from an analogue of modern sea water, dominated by Nd typical of that of the average exposed continental crust (as recorded by fine-grained clastic sediments) but also containing a relatively juvenile component. In no case does Proterozoic BIF show evidence for a dominantly juvenile mantle source for its Nd, so that a special source involving unusual amounts of volcanic Nd input seems to be excluded. A direct mantle source of Nd for Archaean BIF cannot be excluded, however, because of the similarity of Nd in the crustal and mantle reservoirs at that time. There is also no evidence for the derivation of a major component of BIF Nd from pre-3,500 Myr continental sources. If relatively ancient continental crust (>4,000-Myr-old) existed at times of BIF deposition, then not only is its contribution to the clastic sedimentary mass unresolvable, but any contribution it made to major water bodies was masked by inputs from more juvenile crustal sources.

The results of this investigation directly address the source of Nd in BIF and demonstrate the importance of continental sources of Nd in the oceans during the Proterozoic as well as at the present day, but leave open to conjecture the source of Fe. The Fe/Nd ratio of the continental crust and typical clastic sediments is $\sim 1.5 \times 10^3$ and in the range 10^3 – 10^4 for river waters, compared with 10^5 for typical Hamersley BIF containing 35% Fe and 3 p.p.m. Nd. Both Fe and Nd in Precambrian BIF could have been supplied from continental sources if estuaries offered less impediment to iron transfer from rivers to the open ocean than they do today^{62–66}. Although the alternative possibility, a submarine hydrothermal source of Fe in BIF (but not for Nd) is appealing⁶, it is difficult to evaluate. The Fe/Nd ratio of

present-day submarine hydrothermal vent waters is poorly known but apparently high (10^5 – 10^6)^{67–69}, and this was probably always the case. Therefore, if the Fe in BIF was derived from such a source, its Nd budget would probably have been dominated by continental Nd in the surrounding sea water, consistent with observations on Nd in modern metalliferous sediments and hydrogenous deposits¹⁷. In the absence of a more effective tracer for hydrothermally derived iron, it is necessary to consider iron mass-balance. The submarine hydrothermal supply of Fe to the present oceans is estimated to be $\leq 10^{10}$ kg yr⁻¹ (ref. 70), yet it is claimed that the Hamersley BIF alone required $\geq 10^{10}$ kg yr⁻¹ (ref. 5). On the present limited evidence, a major component of Fe derived from the continents may be required to satisfy the iron mass-balance, unless the hydrothermal Fe input was overwhelmingly greater than the present one.

Conclusions

First, Archaean clastic sediments sampled so far were derived from precursors with short previous histories of crustal residence, generally no more than ~200 Myr. Second, Nd in oxide-facies BIF was derived from source material indistinguishable from that supplying the clastic sedimentary mass. A marine source with a Nd budget similar to that of modern oceans is possible for all BIFs, and a dominantly mantle source is excluded for the post-Archaean varieties. The possibility that Fe may also have been derived largely from continental weathering is not excluded. Third, the input of significant amounts of pre-4,000-Myr clastic material into post-3,800-Myr sediments is excluded by the present data. If continental crust existed in significant quantities before 4,000 Myr, then its erosion products were not incorporated into the surviving Archaean sediments and its signature occurs in neither banded iron formations nor, presumably, the water masses from which they were precipitated. Finally, the similarity throughout Earth history of Nd in coeval fine-grained clastic sediments and BIF, and hence between coeval clastic sediments and ocean water, implies that Archaean clastic sediments may be representative of contemporary crust exposed to erosion as are present-day river sediments.

We thank J. Hoefs for samples from Morro do Urucum and J. Veizer and S. L. Goldstein for useful suggestions. This research was supported by NERC and the Royal Society. Department of Earth Sciences Contribution No. 545.

Received 5 September 1984; accepted 30 January 1985.

- Cloud, P. *Geol. Soc. S. Afr. Annurex* **79**, 1–33 (1976).
- James, H. L. in *Iron-Formation: Facts and Problems* (eds Trendall, A. F. & Morris, R. C.) 471–490 (Elsevier, Amsterdam, 1983).
- Walker, J. C. G. et al. in *Earth's Earliest Biosphere* (ed. Schopf, J. W.) 260–290 (Princeton University Press, 1983).
- Riley, J. P. & Chester, R. *Introduction to Marine Chemistry* (Academic, London, 1971).
- Ewers, W. E. in *Iron-formation: Facts and Problems* (eds Trendall, A. F. & Morris, R. C.) 491–512 (Elsevier, Amsterdam, 1983).
- Veizer, J. in *Earth's Earliest Biosphere* (ed. Schopf, J. W.) 240–259 (Princeton University Press, 1983).
- McLennan, S. M. & Taylor, S. R. *Nature* **285**, 621–624 (1980).
- McLennan, S. M. & Taylor, S. R. *J. Geol.* **90**, 347–361 (1982).
- Taylor, S. R. & McLennan, S. M. *Phil. Trans. R. Soc. A* **301**, 381–399 (1981).
- McCulloch, M. T. & Wasserburg, G. J. *Science* **200**, 1003–1011 (1978).
- Goldstein, S. L., O'Nions, R. K. & Hamilton, P. J. *Earth planet. Sci. Lett.* **70**, 221–236 (1984).
- O'Nions, R. K., Hamilton, P. J. & Hooker, P. J. *Earth planet. Sci. Lett.* **63**, 229–240 (1983).
- Hamilton, P. J., O'Nions, R. K., Bridgwater, D. & Nutman, A. P. *Earth planet. Sci. Lett.* **62**, 263–272 (1983).
- Miller, R. G. & O'Nions, R. K. *Earth planet. Sci. Lett.* **68**, 459–470 (1984).
- Frost, C. D. & O'Nions, R. K. *Nature* **312**, 53–56 (1984).
- Allègre, C. J. & Rousseau, D. *Earth planet. Sci. Lett.* **67**, 19–34 (1984).
- Goldstein, S. L. & O'Nions, R. K. *Nature* **292**, 324–327 (1981).
- O'Nions, R. K., Carter, S. R., Cohen, R. S., Evensen, N. M. & Hamilton, P. J. *Nature* **273**, 435–438 (1978).
- Piegras, D. J., Wasserburg, G. J. & Dasch, E. J. *Earth planet. Sci. Lett.* **45**, 223–236 (1979).
- Piegras, D. J. & Wasserburg, G. J. *Earth planet. Sci. Lett.* **50**, 128–138 (1980).
- Tankard, A. J. et al. *Crustal Evolution of Southern Africa* (Springer, Berlin, 1982).
- Eriksson, K. A. *Precamb. Res.* **12**, 141–160 (1980).
- Condie, K. C., Macke, J. E. & Reimer, T. O. *Bull. geol. Soc. Am.* **81**, 2759–2776 (1970).
- Reimer, T. O. *Sediment. Geol.* **7**, 263–282 (1972).
- McLennan, S. M., Taylor, S. R. & Kroner, A. *Precamb. Res.* **22**, 93–124 (1983).
- Hegner, E. et al. *Earth planet. Sci. Lett.* **70**, 267–279 (1984).
- Henderson, J. B. *Bull. geol. Surv. Can.* **246**, 1–62 (1975).
- McGlynn, J. C. & Henderson, J. B. in *Variations in Tectonic Styles in Canada* (eds Price, R. A. & Douglas, R. J. W.) 505–526 (Geol. Ass. Can. Spec. Pap. No. 11, 1972).
- Green, D. C. & Baadsgaard, H. *J. Petrol.* **12**, 177–217 (1971).
- Frith, R. A. & Loveridge, W. D. *Geol. Surv. Can. Pap.* No. 82–1A, 225–237 (1982).
- Moorbath, S., O'Nions, R. K. & Pankhurst, R. J. *Nature* **245**, 138–139 (1973).
- Moorbath, S., Allaart, J. N., Bridgwater, D. & McGregor, V. R. *Nature* **270**, 43–45 (1977).
- Michard-Vitrac, A., Lancelot, J. & Allègre, C. J. *Earth planet. Sci. Lett.* **35**, 449–459 (1977).
- Hamilton, P. J., O'Nions, R. K., Evensen, N. M., Bridgwater, D. & Allaart, J. N. *Nature* **272**, 41–43 (1978).
- Nutman, A. P., Bridgwater, D., Dimroth, E., Gill, R. C. O. & Rosing, M. *Rapp. Grøn. geol. Unders.* **112**, 5–22 (1983).
- Baadsgaard, H. *Rapp. Grøn. geol. Unders.* **112**, 35–42 (1983).
- Baadsgaard, H., Lambert, R. St J. & Krupicka, J. *Geochim. cosmochim. Acta* **40**, 513–527 (1976).
- Bridgwater, D., Keto, L., McGregor, U. R. & Myers, J. S. in *Geology of Greenland* (eds Escher, A. & Watt, W. S.) 18–75 (Geol. Surv. Greenland, Copenhagen, 1976).
- Nielsen, B. L. in *Geology of Greenland* (eds Escher, A. & Watt, W. S.) 460–487 (Geol. Surv. Greenland, Copenhagen, 1976).
- Appel, P. W. U. *Precamb. Res.* **11**, 73–87 (1980).
- Bayley, R. W. & James, H. L. *Econ. Geol.* **68**, 934–959 (1973).
- Morey, G. B. in *Iron-Formation: Facts and Problems* (eds Trendall, A. F. & Morris, R. C.) 13–67 (Elsevier, Amsterdam, 1983).
- Haase, C. S. *Econ. Geol.* **77**, 60–81 (1982).
- Lepp, H. in *Studies in Mineralogy and Precambrian Geology* (eds Doe, B. R. & Smith, D. K.) 265–278 (Geol. Surv. Am. Mem. No. 135, 1972).
- Trendall, A. F. in *Iron-Formation: Facts and Problems* (eds Trendall, A. F. & Morris, R. C.) 69–129 (Elsevier, Amsterdam, 1983).
- Ewers, W. E. & Morris, R. C. *Econ. Geol.* **76**, 1929–1953 (1981).
- Becker, R. H. & Clayton, R. N. *Geochim. cosmochim. Acta* **40**, 1153–1165 (1976).
- Beukes, N. J. *Econ. Geol.* **68**, 960–1004 (1973).
- Burger, A. J. & Coertze, F. J. *Bull. geol. Surv. S. Afr.* **58**, 1–46 (1973).
- Davies, R. D., Allsopp, H. L., Erlank, A. J. & Manton, W. I. *Geol. Soc. S. Afr. Spec. Publ.* **1**, 576–593 (1970).
- Hamilton, P. J., Evensen, N. M., O'Nions, R. K., Smith, H. S. & Erlank, A. J. *Nature* **279**, 298–300 (1979).
- Dorr, J. van N. *Econ. Geol.* **68**, 1005–1022 (1973).
- Hooker, P. J., Hamilton, P. J. & O'Nions, R. K. *Earth planet. Sci. Lett.* **56**, 180–188 (1981).
- McLennan, S. M., Taylor, S. R. & McGregor, V. R. *Geochim. cosmochim. Acta* **48**, 1–13 (1984).
- Schärer, U. & Allègre, C. J. *Can. J. Earth Sci.* **19**, 1910–1918 (1982).
- Nikic, Z. et al. *Geol. Soc. Am. Spec. Pap.* **182**, 169–175 (1980).
- Krogh, T. E. & Gibbins, W. *Geol. Soc. Am. Abstr. Prog.* **10**, 438 (1978).
- Frith, R., Frith, R. A. & Doig, R. *Can. J. Earth Sci.* **14**, 1356–1373 (1977).

59. Nance, W. B. & Taylor, S. R. *Geochim. cosmochim. Acta* **40**, 1539-1551 (1976).
 60. Nance, W. B. & Taylor, S. R. *Geochim. cosmochim. Acta* **41**, 225-231 (1977).
 61. Fryer, B. J. *Geochim. cosmochim. Acta* **41**, 361-367 (1977).
 62. Boyle, E. A., Edmond, J. M. & Sholkovitz, E. R. *Geochim. cosmochim. Acta* **41**, 1313-1324 (1977).
 63. Fox, L. E. & Wofsy, S. C. *Geochim. cosmochim. Acta* **47**, 211-216 (1983).
 64. Fox, L. E. *Geochim. cosmochim. Acta* **48**, 879-884 (1984).
 65. Sholkovitz, E. R. *Geochim. cosmochim. Acta* **40**, 831-845 (1976).
 66. Sholkovitz, E. R., Boyle, E. A. & Price, N. B. *Earth planet. Sci. Lett.* **40**, 130-136 (1978).
 67. Michard, A., Albarède, F., Michard, G., Minster, J.-F. & Charlou, J. L. *Nature* **303**, 795-797 (1983).
 68. Edmond, J. M. *et al. Earth planet. Sci. Lett.* **46**, 19-30 (1979).
 69. Von Damm, K. L. thesis, Massachusetts Institute of Technology (1983).
 70. Boström, K. *Earth planet. Sci. Lett.* **9**, 348-354 (1970).

Transmission and expression of a specific pair of rearranged immunoglobulin μ and κ genes in a transgenic mouse line

Sandro Rusconi^{**} & Georges Köhler^{††}

^{*} Institut für Molekularbiologie II der Universität Zürich, Hönggerberg, CH-8093, Zürich, Switzerland

[†] Basel Institute for Immunology, Grenzacherstrasse 487, CH-4005 Basel, Switzerland

Two genes that code for a hapten-specific immunoglobulin M (IgM) have been introduced into the mouse germ line. The transgenic antibody represents 10-50% of the serum IgM and is expressed on the membrane of B cells. B-cell hybridoma lines show that a negative feedback inhibition of μ and κ transgenic products on the immunoglobulin heavy-chain rearrangement is possible.

EXPRESSION of immunoglobulin genes depends on an accurate rearrangement of DNA to bring several gene segments (for example, variable (V_H), diversity (D_H) and joining (J_H) for the heavy chain and V_L and J_L for the light chain) into juxtaposition¹⁻³. When a complete functional reading unit is achieved in one chromosome, no further rearrangements of the second chromosome occur⁴, a phenomenon termed allelic exclusion. The mechanism responsible for this peculiarity of the immunoglobulin system is poorly understood. To alter the normal rearrangement process, we introduced rearranged μ and κ genes into the germ line of Swiss albino mice. Germline transmission and tissue-specific expression occur in several genes, including immunoglobulin heavy- and light-chain genes⁵⁻¹¹. Here we show transmission and expression of genes encoding a complete antigen-specific antibody molecule.

Transgenic line derivation

The plasmid, microinjected as circular DNA, contains three genes (Fig. 1a); rearranged μ and κ genes encoding an IgM molecule with anti-trinitrophenyl (TNP) specificity (Sp6 in ref. 12) and a bacterial gene for neomycin resistance, the latter under control of the simian virus (SV)40 promoter/enhancer. In gene transfer of B-cell myeloma lines, all the genes of this plasmid are functional¹³.

DNA isolated from the tails of 5 surviving mice obtained from 13 implanted embryos was hybridized to plasmid pBR322 DNA; in one case (209/ F_0 , Fig. 1b) we obtained weak but significant hybridization. Mating the 209/ F_0 male to a random-bred female yielded 10 offspring mice; the DNA from one of them (F_{1a}) hybridizes ~ 10 -fold more strongly with pBR. Now we have obtained 40 offspring of the 209/ F_0 founder, of which three carry the gene. We conclude that 209/ F_0 has chimaeric spermatogonia. Male and female offspring of the F_{1a} male show $\sim 50\%$ segregation (a total of 31⁺/54 in matings with random-bred Swiss Albino ICR/Z, BALB/c and A/J females), as is expected if the plasmid DNA is integrated in one autosomal chromosome.

The presence of pR-HL_{TNP} sequences was checked in a Southern blot analysis where F_0 , F_{1a} and F_2 tail DNA was cut with *Bgl*II and *Pst*I restriction enzymes and probed with an α_1 -globin-containing plasmid. Small 2.5- and 3.5-kilobase (kb)

*Pst*I fragments and a large 11-kb *Bgl*II fragment were revealed, as predicted from the behaviour of the pR-HL_{TNP} plasmid (Fig. 1c, bands marked T). By comparing the intensity of the 'T' bands with endogenous α -globin bands (Fig. 1c), we estimate that there are about one and four copies of pR-HL_{TNP} per diploid genome in 209/ F_0 and F_{1a} (or F_2), respectively. This can be explained by assuming that in F_{1a} (or F_2) every tail cell carries ~ 4 copies of pR-HL_{TNP} (a number confirmed in B hybridomas), but in 209/ F_0 , an average of one cell in four has the same copy number. An analysis of B-cell hybridomas obtained from 209/ F_0 lymph node cells also reveals a mosaic distribution of the transfected gene—only 2 out of 19 hybrids analysed expressed Sp6-like IgM.

Specific IgM

Serum immunoglobulin determinations in transgenic and control mice are summarized in Table 1. All transgenic mice have an elevated anti-TNP/sheep erythrocyte (SRBC) agglutination titre correlating with an elevated expression of an IgM-associated idiotype determinant characteristic of Sp6 IgM. There are also small but significant amounts of Sp6 IgM in the F_0 serum. Quantitative radioimmunoassays, using monoclonal anti- μ for coating and radiolabelled monoclonal anti- μ or anti-idiotypic antibodies to reveal total and idiotypically positive (Id⁺) IgM, showed between 140 and 420 μ g Id⁺-IgM and 380-1,200 μ g total IgM in F_2 and M_2 (mating 2) transgenic adult sera. That is, there is a 1,000-4,000-fold increase of Id⁺-IgM and a 2-5-fold increase of total IgM in transgenic F_2 or M_2 mice over their normal litter mates. The relatively small increase of total IgM may be insignificant or may be unrelated to the transfection, as similar increases do not occur in the sera of transgenic animals resulting from matings of F_{1a} with BALB/c- and A/J females. We found similar levels of protein A binding, κ -light-chain-bearing immunoglobulins (mostly IgG) and λ -light-chain levels in transgenic and control mice (Table 1). Therefore, the introduced IgM does not interfere dramatically with the normal levels of the intrinsic immunoglobulin classes.

To elucidate further the cells responsible for the high Id⁺-IgM concentrations in the serum, we measured immunofluorescence of spleen cells (control values of pR-HL_{TNP}-negative litter mates in parentheses). Approximately 42% (41%) of the cells were stained with an anti- μ reagent and $\sim 27\%$ (3%) and 22% (2%) were double stained with TNP-bovine serum albumin and anti-idiotypic, respectively. This shows that spleen B cells express membrane-bound Id⁺-IgM. To confirm this expression, we

^{††} Present addresses: School of Medicine, Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143, USA (S.R.); Max-Planck Institut für Immunobiologie, Stübeweg 51, Postfach 1169, D-7800 Freiburg, FRG (G.K.).

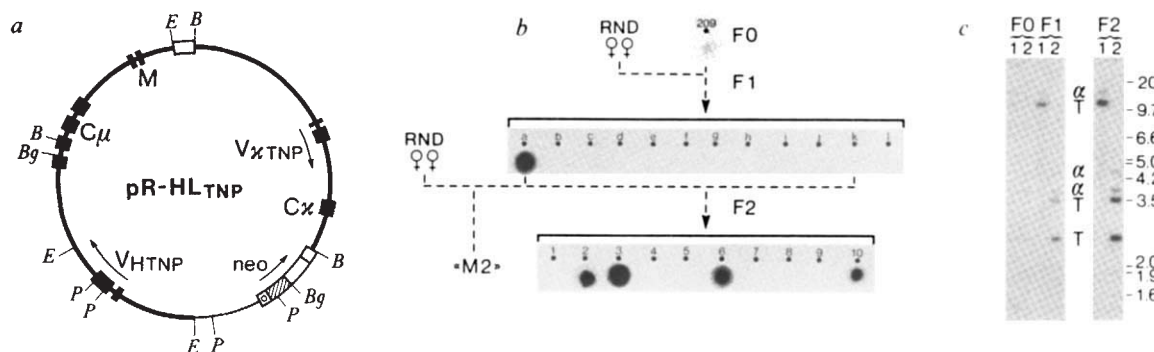


Fig. 1 Transformation of mice with pR-HL_{TNP} DNA. **a**, Schematic representation of the 30-kb DNA used for microinjection, bearing both the functionally rearranged κ light-chain (V_{TNP} and C_{κ}) and heavy-chain (V_{HTNP} and C_{μ}) genes, the combined products of which give rise to an antibody specific for the hapten TNP (see refs 12, 13). Thick line, mouse genomic DNA; thick bars, exons; open bars and hatched bars, SV40 and neomycin resistance sequences from the vector pSV2-neo²⁶; thin line, plasmid DNA. Restriction sites: E, *EcoRI*; B, *BamHI*; only the *BglII* (Bg) and *PstI* (P) sites indispensable for the interpretation of the Southern blot presented in **c** are shown. **b**, The pR-HL_{TNP} transgenic mouse line analysed by dot-blot hybridization. Thirteen fertilized eggs that survived pronuclear microinjection of 20–100 molecules of pR-HL_{TNP} were implanted in the oviduct of a recipient female (see ref. 27). From the five offspring, one contained pBR-hybridizing sequences. The F₁ progeny was generated by mating mouse 209 with a random-bred female. The F₂ progeny resulted from *inter se* cross of mouse F_{1a} and the negative female F_{1k}. Repeated crosses of F_{1a} with other females of the same or different strains lead to the 'M₂' (mating 2) generation (to date, 54 individuals) which was screened either by dot or Southern blot hybridization or serum analysis (see Table 1). Conditions for dot-blot: 2 μ g DNA from the tail tip²⁸ was spotted on nitrocellulose filters (loading buffer, 0.5 M NaOH, 1.5 M NaCl (Schleicher & Schuell)); 0.45 μ m; manipulations essentially as in ref. 29). ³²P-nick translated pBR327 (6×10^8 d.p.m. μ g⁻¹) was used to probe for the presence of exogenous plasmid sequences in the genomic DNA. **c**, Determination of relative copy number and structural organization of the exogenous pR-HL_{TNP} DNA in the transgenic line 209. The DNA was analysed by Southern blots (details in ref. 28). Each lane contains 1 μ g of restricted genomic DNA (1, *BglII*; 2, *PstI*). The hybridization probe was a pBR recombinant clone containing 2.7-kb mouse α_1 -globin DNA³⁰ labelled with ³²P-CTP. Bands resulting from hybridization with the pBR moiety of pRHL_{TNP} are indicated with T; α indicates the fragments which contain α -globin gene sequences and which serve as an internal control (see text).

made B-cell hybridomas, using as fusion partner the Sp2/0-Ag14 line¹⁴.

B-cell hybridomas

Lipopolysaccharide (LPS)-stimulated lymph node cells of the F_{1a} mouse yields 32 of 72 wells with B-cell hybridoma growth, 30 of which contain anti-TNP-specific Id⁺-IgM in the supernatant. Supernatants of radiolabelled cultures of independent recloned hybridoma lines were analysed by SDS and isoelectric focusing (IEF) polyacrylamide gel electrophoresis (PAGE) under reducing conditions (Fig. 2; Table 2). Only those hybrids originating from F_{1a} lymph node cells and listed in Table 2 will be discussed here in detail.

For 5 of the 11 F_{1a} hybrids analysed (for example, C5-20-21 and C5-20-22; Fig. 2) a doublet of light-chain-like bands, the weaker of which co-migrated with the Sp6 light chain, was seen after SDS-PAGE. The other six lines (for example E5-28-48 in Fig. 2) show only one strong band at the Sp6 light-chain position. To demonstrate that low concentrations of $\kappa 6$ are co-expressed with newly rearranged light chains, IEF-PAGE was used. Although $\kappa 6$ is not seen easily (Fig. 2), it is visible in the original autoradiogram and all lines express it in addition to a cell-line-specific light chain (Fig. 2, arrows). Light chains are easily identified in IEF-PAGE under reducing conditions as isolated bands (heavy chains always run as a series of closely spaced bands). A series of at least three bands characteristic of the Sp6 μ -heavy chain is expressed at varying intensity by all hybrids analysed except H5-35. In addition, in some hybrids, a new set of bands is seen (Fig. 2), which suggests that there is co-expression of μ -heavy chains. This co-expression was confirmed by the subclone C5-20-16, which has lost the pR-HL_{TNP} DNA (not shown) together with the $\kappa 6$ light chain and idiotype expression, but which synthesizes an IgM with a 20-fold lower TNP/SRBC agglutination titre (Fig. 2; Table 2). Surprisingly, the IEF-bands co-migrating with Sp6 μ -chain are preserved in this line.

Sp6-encoded RNA

To confirm the expression of Sp6 μ - and κ -chains, we performed Northern blot analysis of cytoplasmic hybridoma and Sp6 RNA using $V_{\mu 6}$ and $V_{\kappa 6}$ DNA probes (Fig. 3, Table 2). All

lines express the expected 2.4- and 1.2-kb RNA when probed with $V_{\mu 6}$ and $V_{\kappa 6}$, respectively. To quantify the fraction of μ and κ RNA associated with $V_{\mu 6}$ and $V_{\kappa 6}$ variable regions, we performed RNA dot-blot experiments. RNA was blotted on nitrocellulose filters at different concentrations and equivalent samples were hybridized to radiolabelled probes specific for the four elements $V_{\mu 6}$, μ constant region (C_{μ}), $V_{\kappa 6}$ and C_{κ} . The radioactive signals were evaluated quantitatively by scintillation counting of the corresponding excised filter portions (Fig. 3). In all four hybridomas, the intensity of the $V_{\kappa 6}$ signal is about one-quarter of that of Sp6, whereas the C_{κ} signal is 1.4–4.6 times stronger than that of Sp6. Thus there is a 6–20-fold excess of C_{κ} over $V_{\kappa 6}$ in the four hybridomas analysed. We conclude

Table 1 Immunoglobulin levels of transgenic mice

Serum	TNP/SRBC agglutination titre	Idiotype (μ g ml ⁻¹)	IgM (μ g ml ⁻¹)	Protein A binding (mg ml ⁻¹)	λ ratio Id ⁺ /Id ⁻
F ₀	30	0.2	150	2.7	ND
F _{1a}	5,000	200	1,000	2.7	ND
F _{2/3}	8,000	220	460	2.0	
F _{2/6}	8,000	420	1,200	2.5	1
C2 + C4 pool (F ₂ littermates)	<10	<0.1	240	1.3	
M ₂ /40					
41	3,000	140	380	1.0	
42					1
M ₂ /43 + 44 pool (M ₂ littermates)	<10	<0.1	280	2.0	

Radioimmunoassays were performed by incubating serially diluted sera into antibody-coated 96-well polyvinyl plates. To measure idiotype and IgM titres, plates were coated with a monoclonal anti-mouse μ antibody (C2-23; ref. 23), bound material was revealed with a ¹²⁵I-labelled anti-idiotypic monoclonal antibody (20-5; obtained from P.A. Cazenave) or a second, anti-mouse μ antibody (M41; ref. 23). Absolute amounts were obtained by comparison with purified Sp6 IgM. To measure protein A binding material, plates were coated with protein A and bound material was revealed with an anti-mouse κ monoclonal antibody (187.1; ref. 24). Reference material came from C57BL/6 serum purified on a protein A column. Relative amounts of λ -chains in sera of transgenic against normal mice were measured by coating the plates with an anti-mouse λ antibody (L22.14.4; ref. 25) and revealing bound material with the same ¹²⁵I-labelled antibody. Agglutination titres on TNP-coupled SRBC (TNP/SRBC) were enhanced with C2-23 antibody. ND, not done.

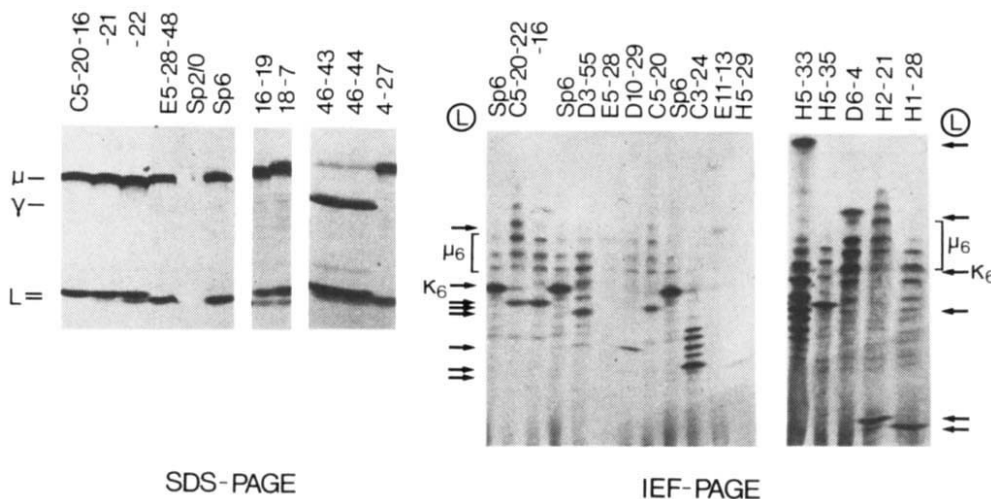


Fig. 2 Separation of reduced immunoglobulin chains. ^{14}C -labelled culture supernatants were obtained and analysed by SDS and IEF-PAGE as described previously¹². Arrows indicate the positions of endogenously rearranged light chains (IEF-PAGE). Supernatants of lines 16-19, 18-7, 46-43, 44 and 4-27 come from a different fusion and are included to demonstrate co-expression of μ - and γ -chains (46-43, 44) and of λ 1- and κ 6-chains (18-7). Antibodies from these lines are TNP- and idiotype-positive.

that, at least in the four hybridomas analysed, the Sp6-derived light chain is expressed at about one-tenth of the level of the second (endogenous) κ light chain. A similar analysis of V_{μ} 6-carrying μ RNA reveals that in C5-20, ~40%, and in the other hybrids, ~75% of μ RNA might be derived from Sp6.

Heavy-chain locus rearrangements

In 7 out of 11 hybrids, there is no evidence for co-expression of a μ -chain derived from Sp6 with an endogenously derived μ -chain (Table 2). To exclude chromosome loss and to test for rearrangements in the J_H region, we hybridized the *Pvu*II- and *Xba*I-restricted DNA from all clones with a purified J_H3/J_H4 (*Bam*HI/*Eco*RI) probe (Fig. 4a; Table 2), with DNA from the Sp2/0 fusion partner and BALB/c liver as controls.

The Sp2/0 band is present in the DNA of all cloned hybridomas except D10-29 and H5-33. The hybridomas share the following properties: they show one strong and two weaker pR-HL_{TNP}-derived bands (dots, Fig. 4a); they have lost the germline J_H band of 4.8 kb (*Pvu*II) and 3.7 kb (*Xba*I) but have replaced it with one or two weaker bands of varying size. We conclude that all hybrids have retained both the chromosome carrying the pR-HL_{TNP} DNA and also either one or both chromosomes encoding the heavy-chain genes (Table 2).

DNA of the hybrid cells, cut with *Pvu*II and *Xba*I restriction enzymes, were also hybridized to two different 5' *D*-segment probes (Fig. 4b; Table 2). If a heavy-chain gene is rearranged to become a complete *V-D-J* unit, the intervening DNA containing the *D* segment is deleted and can no longer be detected with 5' *D* probes⁴. The DNA of the Sp6 line has two rearranged J_H loci¹⁵, both of which are of the *V-D-J* type, as no band is hybridized with the *D* probes (Fig. 4b). The Sp2/0-fusion parent has one rearranged J_H locus in the *D-J* configuration, as most of the germline *D* bands are revealed.

The same rearranged band which has been revealed with the *J3/J4* probe at ~17 kb (*Pvu*II) is seen also with the *D* probe in H10, D3-55 and E5-28. Furthermore, as seen in the DNA cut with *Pvu*II, both *J3/J4*-containing bands of E5-28 (22 and 17 kb) and D10-29 (11 and 6.5 kb) hybridize with the *D* probe. In the E5-28 line, the 22-kb DNA (*Pvu*II) that contains *J3/J4* is not seen easily when hybridized to the *D* probe, it is better visualized in the 3.8 kb band appearing after *Xba*I digestion (the 17-kb *Pvu*II band is hidden by other bands in this digest). Thus, in the E5-28 and D10-29 lines, rearrangements have stopped at both heavy-chain loci at the *D-J* stage, and no endogenous μ -chain could be made. In Table 2 the number of *J* and *D* rearrangements are given for all hybrids analysed.

Table 2 Summary of the characterization of 209/F_{1a} B-cell hybridomas

	Anti-TNP titre 3 ⁿ	IEF			IgM (μg ml ⁻¹)	Id ⁺ (μg ml ⁻¹)	Presence of insert DNA	mRNA V _μ 6 V _κ 6		J _H probe No. of bands detected	D probe No. of J-bands detected	Possible protein	Phenotype DNA heavy-chain	
	n	μ6	κ6	L	SDS									
Sp6	8	+	+	-	μ6, κ6	10	10	-	+	+	2	0	μ6κ6	VDJ ⁺ , VDJ ⁻
1 D3-55	4	+	+	+	μ, κ	14	2	+	+	+	2	1	μ6κ6/κ	VDJ ⁻ , DJ
2 E5-28	3	+	+	+	μ, κ	5	1	+	+	+	2	2	μ6κ6/κ	DJ, DJ
3 D10-29	2	+	+	+	μ, κ	4	0.7	+	+	+	2	2	μ6κ6/κ	DJ, DJ
4 C5-20	3	*	+	+	μ, κ, κ6	3.5	0.9	+	+	+	2	0	μ6κ6/μ, κ	VDJ ⁺ , VDJ ⁻
5 H2-21	Nil	*	+	+	μ, κ, κ6	4.5	0.8	+	+	+	1	1	μ6κ6/κ	DJ
6 H1-28	4	+	+	+	μ, κ, κ6	2	0.8	+	+	+	1	1	μ6κ6/κ	DJ
7 E11-13	7	+	+	+	μ, κ	2.5	1.4	+	+	+	2	1	μ6κ6/κ	DJ, germ line
8 H5-33	Nil	*	+	+	μ, κ	10	1.5	+	+	+	2	1	μ6κ6/μ, κ	VDJ ⁺ DJ
9 H5-29	1	Low	+	+	κ, (κ6)	0.04	0.08	+	+	+	1	1	(μ6κ6)/κ	DJ
10 C3-24	4	*	+	+	μ, κ, κ6	2	0.6	+	+	+	2	0	μ6κ6/μ, κ	VDJ ⁺ /VDJ ⁻
11 D6-4	6	+	+	+	μ, κ	ND	ND	+	ND	ND	2	2	μ6κ6/κ	DJ/DJ
C5-20-22	6	As C5-20				12	6	+	+	+	2	ND	μ6κ6/μ, κ	
C5-20-16	3	+	-	+	μ, κ	7	<0.04	-	-	-	2	ND	μ, κ	
D3-55-19-60	5	As D3-55				30	11	+	ND	ND	2	ND	μ6κ6/κ	
D3-55-19-63	Nil	-	-	-	-	-	-	-	ND	ND	2	ND	Nil	
E11-34-46	8	As E11-13				30	10	+	ND	ND	2	ND	μ6κ6/κ	
E11-34-47	Nil	-	-	+	κ	-	-	-	ND	ND	2	ND	κ	

TNP/SRBC agglutination, IgM and idiotype (Id⁺) titres were measured on supernatants of the indicated lines as described for Table 1. For IEF, + indicates a band or a series of bands co-migrating with κ_6 or μ_6 in the presence of an endogenously rearranged second light (L) chain; SDS pattern, presence of material running at the positions of the μ and κ is indicated; κ_6 indicates the presence of a second light-chain band co-migrating with the Sp6 κ -chain. Possible phenotypes are written for proteins as μ_6 and κ_6 representing Sp6-like IgM, μ and κ representing additional endogenous chains. Among the rearrangements at the J_H locus, *V-D-J*⁻ indicates a putatively inactive rearrangement. In H2-21, co-expression of two heavy chains is implied by the Id⁺/TNP⁻ phenotype and the IEF pattern, but we were unable to reveal a second J_H -containing DNA band using seven restriction enzymes. In E11-13, using seven restriction enzymes, a J_H -containing DNA band co-migrating with the germ line J_H band was found. ND, not done. * Additional bands present.

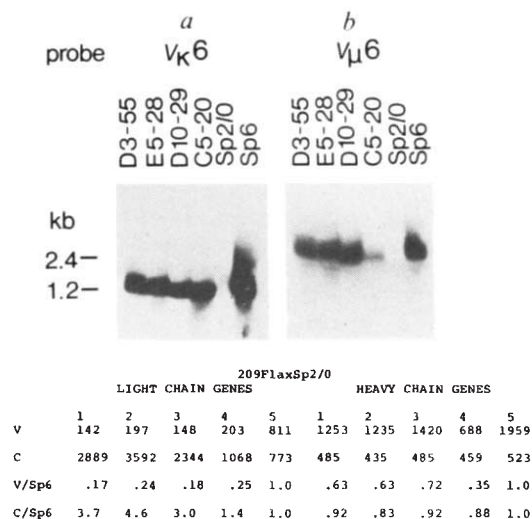


Fig. 3 Northern blots on RNA from B-cell hybridomas. Total cytoplasmic RNA (30 μ g) was separated on agarose gels as described previously³¹. The filters were probed with ³²P-labelled pBR322 plasmid DNA ($2-8 \times 10^8$ c.p.m. μ g⁻¹) containing the entire V κ 6 (a) or V μ 6 (b) region. Purified fragments containing V κ 6, C κ and V μ 6 coding sequences and pAB μ -1 containing C μ (ref. 32) were used to derive the quantitative estimates listed below. Numbers 1-5 correspond to lines D3-55, E5-28, D10-29, C5-20 and Sp6, respectively.

Table 2 also shows the deduced organization of their heavy-chain loci, as well as the putative expression of endogenous heavy and light chains. A V-D-J⁻ rearrangement is defective for μ protein production. This is shown in D3 by the failure of μ -chain production in its subclone (which had lost the pRHL_{TNP} DNA) (Table 2). The V-D-J⁻ alleles of C5-20 and C3-24 were assumed to be defective, as no evidence of a third μ -chain was found by IEF-PAGE.

Discussion

Our analysis shows that not only μ (11) and κ (9) genes separately but also both together can be introduced into the mouse germ line and expressed in an orderly fashion in B cells (IgM membrane deposition, secreted pentameric and functional IgM in serum). A third gene, *neo*, encoding neomycin resistance and under SV40 promoter/enhancer control, does not protect hybrid cells from the toxicity of the neomycin analogue G418, nor do we find *neo*-specific cytoplasmic RNA of the correct size (data not shown). It remains to be seen whether this gene is still intact in transgenic mice.

In normal mice, a cell never expresses more than one heavy-chain product or more than one light-chain product^{4,16}. This allelic exclusion is thought to be necessary for effective clonal selection. Expression of the Sp6 light chain in the B hybridomas was at about one-tenth the level of the co-expressed light chain, and no inhibition of intrinsic light-chain rearrangement was observed. This may be possible because an excess of transgenic μ -chain mRNA and protein occurs in the same cell, thus mimicking the situation in normal B-cell development, where μ appear first¹⁷.

Ritchie *et al.*¹⁸ found that light-chain rearrangement is inhibited when both endogenous μ and the transgenic light chain are synthesized in quantities comparable with those in hybridomas derived from normal mice. Our results, on the other hand, indicate that μ -light-chain molecules in low concentrations with an excess of free heavy chains do not have a measurable effect on the process of light-chain gene rearrangement. Thus, the existence of μ light-chain molecules *per se* is not important in the regulation of the V_L-J_L joining mechanism. It does not contradict models in which the amount of light chain is essential in inhibiting rearrangement¹⁹.

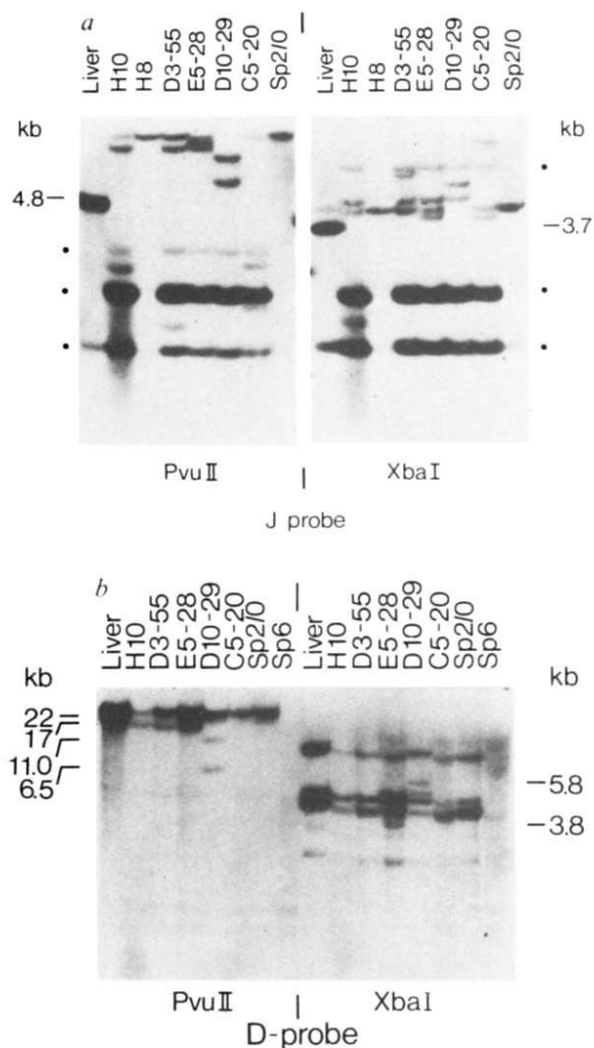


Fig. 4 Joining configuration at the J_H-locus analysed by Southern blotting. a, J probe; b, D probe. DNA (15 μ g) from the indicated B-cell hybridomas was separated on 1% agarose gels and blotted onto nitrocellulose filters, which were then hybridized to nick-translated, purified DNA probes. The BamHI/EcoRI fragment containing the J_H3/J_H4 segment was purified from a pBR322 plasmid containing all four mouse J_H segments (gift of M. Steinmetz). The J_H probe was labelled to 5×10^8 c.p.m. μ g⁻¹ and was used in the blot shown in a. The PstI/PstI fragment containing the 5' DSp2 segment was purified from the plasmid p4-7-5 (ref. 4; gift of M. Reth). The 5' DSp2 probe was labelled to 6×10^6 c.p.m. μ g⁻¹ and was used in the blot in b. Cutting the DNA of H10, D3-55 and E5-28 with both PvuII or XbaI restriction enzymes reveals a common band hybridizing to the J_H probe, also visualized using the 5' DSp2 probe (PvuII, 17 kb). This band is hidden in the series of bands seen between 3.8 and 5.8 kb after XbaI digestion. The 3.8-kb band of E5-28 DNA (XbaI) therefore reflects the DNA coming from the 22-kb (PvuII) band, which is difficult to reproduce in the photograph. All DNA samples were also tested with DFI-16, representative of a second D'-family⁴ (data not shown).

Table 3 Comparison of the rearrangement of J_H alleles of various sources

	V-D-J ⁺	V-D-J ⁻	D-J (%)	Germ line	Total
Hybrids (this paper)	3	3	12(63)	1	19
Abelson* fetal liver	1	7	18(69)	0	26
Abelson* bone marrow	9	15	7(23)	0	31
Myeloma* silent allele	—	20	12(38)	0	32

* From ref. 4.

At the heavy-chain locus, co-expression of μ -chains occurs in the C5-20 line; the variant C5-20-16, which has lost pR-HL_{TNP}, still synthesizes μ -chain (Fig. 2). Co-expression is also probable in lines H5-33, C3-24 and the γ -chain-expressing lines 46-43 and 44 (Fig. 2, Table 2). The amounts of μ 6 produced are low in at least three of the four lines (H5-33, C3-24 and 46-44; Fig. 2). The amounts of μ 6 produced by C5-20 could, however, be approximately equivalent to the amount of the endogenously produced μ -chain). In the λ -expressing 18-7 line, μ 6 and endogenous μ are made in about equal amounts (data not shown). Nevertheless, of 11 hybrids analysed in detail, 8 express μ 6 alone, and as 5 of these retain both heavy-chain loci, chromosome loss in the hybridoma could not account for loss of endogenous μ -chain production (Table 2). By isolation of Sp6/IgM-negative variants of the D3 and E11 lines we have confirmed that there is no endogenous μ production (Table 2).

One of the 19 heavy-chain loci analysed here is arrested in the germline configuration (E11-13), 12 remain in the immature D-J configuration and 6 complete the entire V-D-J coding unit, even though only 3 of them actually produce μ -chains.

Compared with Abelson murine leukaemia virus-transformed bone marrow or myeloma B-cell lines, the transgenic mouse has a high frequency of cells in the immature joining configuration

(Table 3). There are two possible explanations: first, B cells that express Sp6 IgM early in their development might be stimulated by LPS and thereby hybridize earlier than normal B cells. On fusion, the rearrangement machinery is inactivated so that their DNA is frozen in the immature configuration. If, however, light-chain follows heavy-chain gene rearrangement²⁰⁻²², this possibility seems unlikely, as all hybrids express an endogenous light chain. Second, early expression of Sp6 IgM might slow down directly the process of J_H rearrangement. As most lines have made the D-J_H rearrangement, this step must be less vulnerable to inhibition, either because D to J joining precedes competence of a B cell for μ expression or because immunoglobulin chains do not greatly affect this step.

Our results agree with models postulating negative feedback control mechanisms of μ -chain on the process of J_H rearrangement⁴. They do not support a mechanism in which allelic exclusion at the heavy-chain locus is explained by elimination of B cells having two μ -producing rearrangements¹⁹.

We thank Carla Jacot-Guillarmod and Beatrice Dolder for technical assistance, Luciana Forni for the immunofluorescence analysis and C. Steinberg for critically reading the manuscript. The Basel Institute for Immunology is supported by F. Hoffmann-La Roche & Co. Ltd.

Received 26 November 1984; accepted 12 February 1985.

1. Tonegawa, S. *Nature* **302**, 575-581 (1983).
2. Adams, J. M. *et al. Immun. Rev.* **59**, 5-32 (1981).
3. Honjo, T. *et al. Immun. Rev.* **59**, 33-67 (1981).
4. Alt, W. F. *et al. EMBO J.* **3**, 1209-1219 (1984).
5. Constantini, G. & Lacy, E. *Nature* **294**, 92-94 (1981).
6. Wagner, E. F., Stewart, T. A. & Mintz, B. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5016-5020 (1981).
7. Palmiter, R. D., Chen, H. Y. & Brinster, R. L. *Cell* **29**, 701-710 (1982).
8. Palmiter, R. D., Norstedt, G., Gelinas, R. E., Hammer, R. E. & Brinster, R. L. *Science* **222**, 809-814 (1983).
9. Brinster, R. L. *et al. Nature* **306**, 332-336 (1983).
10. Storb, U., O'Brien, R. L., McMullen, M. D., Gollahon, K. A. Brinster, R. L. *Nature* **310**, 238-241 (1984).
11. Grosschedl, R., Weaver, D., Baltimore, D. & Constantini, F. *Cell* **38**, 647-658 (1984).
12. Köhler, G. & Milstein, C. *Eur. J. Immun.* **6**, 511-519 (1976).
13. Ochi, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 6351-6355 (1983).
14. Shulman, M., Wilde, D. C. & Köhler, G. *Nature* **276**, 269-270 (1978).
15. Köhler, G., Potash, M. J., Lehrach, H. & Shulman, M. *EMBO J.* **1**, 555-563 (1982).
16. Alt, F. W., Enea, V., Bothwell, A. L. M. & Baltimore, D. *Cell* **21**, 1-12 (1980).
17. Kincade, P. W. *Adv. Immun.* **31**, 177-245 (1981).
18. Ritchie, K. A., Brinster, R. L. & Storb, U. *Nature* **312**, 517-520 (1984).
19. Wabl, M. & Steinberg, C. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6976-6978 (1982).
20. Alt, F. W., Rosenberg, N., Lewis, S., Thomas, E. & Baltimore, D. *Cell* **27**, 381-390 (1981).
21. Perry, R. P., Kelley, D. E., Coleclough, C. & Kearney, J. K. *Proc. natn. Acad. Sci. U.S.A.* **78**, 247-251 (1981).
22. Korsmeyer, S. S. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 7096-7100 (1981).
23. Leptin, M. *et al. Eur. J. Immun.* **14**, 534-542 (1984).
24. Yelton, D. E., Desaymard, C. & Scharff, M. D. *Hybridoma* **1**, 5-11 (1981).
25. Weiss, S., Lehmann, K. & Cohn, M. *Hybridoma* **2**, 49-54 (1983).
26. Southern, E. M. & Berg, P. *J. molec. appl. Gen.* **1**, 327-341 (1982).
27. Rusconi, S. in *The Impact of Gene Transfer Techniques in Eucaryotic Cell Biology* (eds Schell, J. & Starlinger, P.) (Springer, New York, in the press).
28. Brinster, R. L. *et al. Cell* **27**, 223-231 (1981).
29. Kafatos, F. C., Jones, C. W. & Efstratiadis, A. *Nucleic Acids Res.* **7**, 1541-1552 (1979).
30. Rusconi, S. *Cell* (submitted).
31. McMaster, G. K. & Carmichael, G. G. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4835-4838 (1977).
32. Alt, F. W. *et al. Cell* **20**, 293-301 (1980).

LETTERS TO NATURE

Gravitational radiation from a solid-crust neutron star

M. Ali Alpar & David Pines

Department of Physics, University of Illinois at Urbana-Champaign, 1110 W. Green Street, Urbana, Illinois 61801, USA

The measured period derivative¹⁻³ for the millisecond pulsar PSR1937+21⁴ is $\dot{P} \approx 1.05 \times 10^{-19}$, whereas the present upper limit⁵ for PSR1953+29⁶ is $\dot{P} < 4.2 \times 10^{-17}$. For electromagnetic braking, the combination of rapid rotation with such small period derivatives requires a small ($\sim 10^8$ G) magnetic field. A natural association between rapid rotation and small magnetic field is provided by accretion spin-up scenarios⁷⁻⁹. Here we address the analogous problem for gravitational radiation. Balancing gravitational radiation power against the rate of rotational energy loss, we find that if the effective triaxiality ϵ_{eff} exceeds $\epsilon_{\text{max}} \sim 10^{-9}$, gravitational radiation alone would spin a millisecond pulsar down at a faster rate than the observed $\dot{P}$. We show that $\epsilon_{\text{eff}} \ll \epsilon_{\text{max}}$, so that gravitational radiation is not likely to play a major role in the evolution of millisecond pulsars even in the absence of damping. Damping times for precession of the solid crust are estimated to be $< 2 \times 10^3$ yr.

The energy loss rate due to gravitational radiation is

$$I\Omega\dot{\Omega} = \frac{32}{5} \frac{G}{c^5} I^2 \epsilon_{\text{eff}}^2 \Omega^6 \quad (1)$$

where I is the moment of inertia, Ω the angular velocity and ϵ_{eff} the effective triaxiality. With P and $\dot{P}$ values similar to those for PSR1937+21,

$$\epsilon_{\text{max}} = 1.9 \times 10^{-9} \left(\frac{\dot{P}_{-19}}{I_{45}} \right)^{1/2} P(\text{ms})^{3/2} \quad (2)$$

which seems to impose a constraint on millisecond pulsars as their rotational oblateness, $\epsilon_{\Omega} \equiv I\Omega^2/4A$, is $\sim 10^7$ times larger (see Table 1).

The moment of inertia tensor of a neutron star with a solid crust is

$$\tilde{I} = I_0 \left[\left(1 - \frac{\epsilon_{\Omega}}{2} - \frac{b\epsilon_0}{2} \right) \tilde{e} + \frac{3}{2} \epsilon_{\Omega} \hat{n}_{\Omega} \hat{n}_{\Omega} + \frac{3}{2} b\epsilon_0 \hat{n}_0 \hat{n}_0 \right] \quad (3)$$

where I_0 is the moment of inertia in the spherical case (no rotation), ϵ_{Ω} the 'rotational oblateness', ϵ_0 the 'reference oblateness' of the solid crust, b is a coefficient describing its rigidity, $\hat{n}_0$ and $\hat{n}_{\Omega}$ are unit vectors along the reference axis and the instantaneous angular velocity vector $\tilde{\Omega}$, and $\tilde{e}$ is the unit tensor¹⁰. Note that the triaxiality of $\tilde{I}$ stems from b , the rigidity of the solid. The corresponding fluid star ($b=0$) would have

an axially symmetric equilibrium shape. (Wagoner¹¹ finds that non-axially symmetric perturbations of a fluid neutron star can grow only at angular velocities 1.5–2 times faster than that of PSR1937+21. Friedman¹² finds that non-axial perturbations are stable at $P \approx 1.5$ ms for some equations of state; this result, however, rests on the assumption that the viscosity is that appropriate for a temperature $T \sim 10^9$ K that is far too high for the quite-old millisecond pulsars. We thus conclude that the underlying fluid equilibrium shape is axially symmetric.)

The $\hat{n}_\Omega \hat{n}_\Omega$ term in $\tilde{I}$ acts like the unit tensor $\tilde{e}$ on the $\tilde{\Omega}$ vector. The kinetic energy, T , then has the same form as that of a symmetric top with symmetry axis $\hat{n}_0$

$$T = \frac{1}{2} I_0 \left[(1 + \varepsilon_\Omega + b\varepsilon_0) \Omega_3^2 + \left(1 + \varepsilon_\Omega - \frac{b\varepsilon_0}{2} \right) \Omega_1^2 + \left(1 + \varepsilon_\Omega - \frac{b\varepsilon_0}{2} \right) \Omega_2^2 \right] \quad (4)$$

where Ω_3 is the component of $\tilde{\Omega}$ along $\hat{n}_0$. Then $\hat{n}_0$, $\tilde{\Omega}$ and the angular momentum $\tilde{L}$ all lie in a plane, which precesses about the fixed $\tilde{L}$ axis, with a precession frequency in the non-rotating frame given by¹³

$$\Omega_{pr} = \frac{L}{I_1} = \frac{L}{I_0} \left(1 - \varepsilon_\Omega + \frac{b\varepsilon_0}{2} \right) \approx \Omega \left(1 + \frac{3b\varepsilon_0}{2} \cos^2 \theta_0 \right) \quad (5)$$

where θ_0 is the angle between $\tilde{\Omega}$ and $\hat{n}_0$. We have a triaxial body, which acts dynamically like a symmetric top because the rotationally induced contribution to the non-sphericity of $\tilde{I}$ has $\tilde{\Omega}$ itself as a symmetry axis. To diagonalize $\tilde{I}$, a rotation in the $(\tilde{L}, \tilde{\Omega})$ plane is required. As the two independent vectors $\hat{n}_0$ and $\hat{n}_\Omega$ lie in this plane, so do two principal axes, $\hat{n}_1$ and $\hat{n}_2$. The $(\tilde{L}, \tilde{\Omega})$ plane is a symmetry plane of the triaxial neutron star, which therefore precesses about $\tilde{L}$ at the frequency Ω_{pr} , so that this is also the observed pulsar frequency for a pulsar beam fixed in the body frame.

The gravitational radiation power in a frequency ω is¹⁴

$$P(\omega) = \frac{2G\omega^6}{5c^5} \{ D_{ij}^*(\omega) D_{ij}(\omega) - \frac{1}{3} |D_{ii}(\omega)|^2 \} \quad (6)$$

where

$$D_{ij}(t) = \sum_{\omega > 0} \exp(-i\omega t) D_{ij}(\omega) + \text{complex conjugate} \quad (7)$$

is the moment

$$D_{ij}(t) = M(t)_{ia} M(t)_{jb} \int d^3x' x'_a x'_b \rho(x') \\ = M(t)_{ia} M(t)_{jb} \left(\frac{\text{tr } I}{2} \delta_{ab} - I_{ab} \right) \quad (8)$$

Here $\rho(x')$ is the mass density, $\mathbf{x}'$ is the position vector in the body frame defined by the principal axes and $M(t)$ is the instantaneous transformation matrix from the body frame $\hat{n}_1(t)$, $\hat{n}_2(t)$, $\hat{n}_3(t) \times \hat{n}_1(t)$ to a fixed frame, whose $\hat{x}_3$ axis we choose to be parallel to $\tilde{L}$ (see Fig. 1). Gravitational radiation comes out in the frequencies Ω_{pr} and $2\Omega_{pr}$. Diagonalizing $\tilde{I}$ to find $\hat{n}_1(t)$, $\hat{n}_2(t)$, $\hat{n}_3(t) \times \hat{n}_1(t)$ and thus the matrix $M(t)$, equations (3)–(8) yield the output power, to lowest order in $b\varepsilon_0$ and $b\varepsilon_0/\varepsilon_\Omega$

$$P(2\Omega_{pr}) \approx \frac{72}{5} \frac{GI_0^5}{c^5} \Omega_{pr}^6 b^2 \varepsilon_0^2 \sin^4 \theta_0 \quad (9)$$

$$P(\Omega_{pr}) \approx \frac{9}{10} \frac{GI_0^2}{c^5} \Omega_{pr}^6 (1 - \frac{3}{2} \varepsilon_\Omega)^2 b^2 \varepsilon_0^2 \cos^2 \theta_0 \sin^2 \theta_0 \quad (10)$$

Comparing with equation (1)

$$\varepsilon_{\text{eff}}^2 = (\frac{3}{2} b\varepsilon_0 \sin^2 \theta_0)^2 + (\frac{3}{8} b\varepsilon_0 (1 - \frac{3}{2} \varepsilon_\Omega) \cos \theta_0 \sin \theta_0)^2 \quad (11)$$

These are essentially the well-known results for a precessing axially symmetric body¹⁵. The rotational oblateness $\varepsilon_\Omega \hat{n}_\Omega \hat{n}_\Omega$ contributes to higher order corrections.

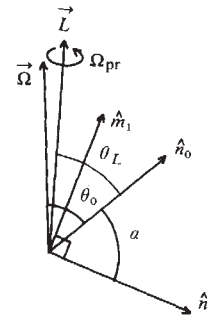


Fig. 1 The reference plane containing $\tilde{L}$, $\tilde{\Omega}$, $\hat{n}_0$ and the principal axes $\hat{n}_1$ and $\hat{n}_2$ of the triaxial solid-crust neutron star. This plane precesses at the rate Ω_{pr} around the angular momentum axis $\tilde{L}$. We have $\theta_L = \theta_0(1 - O(b\varepsilon_0))$ and $\alpha = \pi/2 - \theta_0(1 - O(b\varepsilon_0/\varepsilon_\Omega))$.

We now consider the values of b , ε_0 and θ_0 for a millisecond pulsar that has been spun up by accretion and has been spinning down as a pulsar since the end of the accretion phase⁷.

(1) The rigidity, b . A solid with oblateness, ε , and reference oblateness, ε_0 , has an elastic energy $B(\varepsilon - \varepsilon_0)^2$; the oblateness increases the gravitational potential energy by an amount $A\varepsilon^2$ above the gravitational energy of a spherical star of the same mass. The coefficient b in equation (3) is defined by $b \equiv B/(A + B)$ and Pandharipande, Pines and Smith¹⁶ have calculated A and B for a variety of neutron star models. Typical values imply $b \sim 10^{-5}$.

(2) The reference oblateness ε_0 . ε_Ω is the equilibrium oblateness of a fluid body rotating at angular velocity Ω , $\varepsilon_\Omega \equiv I_0 \Omega^2 / 4A$. The reference oblateness ε_0 is defined as the oblateness of the star at the time of formation of its solid crust, modified subsequently according to its seismic history, through plastic flow or starquakes^{17,18}. If we make the conservative assumption that a neutron star spun up by accretion has, at the time of crust solidification, a period of ~ 1 s, the initial value of the reference oblateness is $\varepsilon_0(0) \approx I_0 \Omega_0^2 / 4A \sim 10^{-6}$. Neglecting plastic flow associated with the logarithmic creep of dislocations in the crust¹⁸, ε_0 will change only through starquakes, which take place when $|\varepsilon_\Omega - \varepsilon_0| \approx \theta_m$, where θ_m , the critical strain angle of the solid crust, is $\sim 10^{-4}$ – 10^{-5} for typical terrestrial metals, whereas for a pure Coulomb lattice, which may be a better approximation to the neutron star crust¹⁹, it is $\sim 10^{-1}$ – 10^{-2} . We consider various possibilities for the evolution of ε_0 : (i) $\theta_m \sim 10^{-1}$. ε_0 follows the accretion spin-up and the subsequent pulsar spin-down by a series of quakes, starting at a time when $\varepsilon_\Omega \sim 10^{-4}$, $\varepsilon_0 \sim 10^{-6}$, to its present value $\varepsilon_0 \approx \varepsilon_\Omega \approx 10^{-1}$ (PSR1937+21) within $\theta_m \sim 10^{-4}$ of ε_Ω . Even if the reference oblateness, ε_0 , were also affected by solidification of accreted material, our above conclusion would not change, as the smallness of the critical angle, θ_m , and the consequent large number of quakes during the $\sim 10^8$ -yr accretion era and the subsequent and comparably long pulsar era will bring the final ε_0 close to ε_Ω . (ii) $\theta_m \sim 10^{-1}$. The reference oblateness, ε_0 , may keep its initial value of $\sim 10^{-6}$ through the entire spin-up era, without reaching the condition $|\varepsilon_\Omega - \varepsilon_0| \approx \theta_m$ for quakes. The subsequent decrease of ε_Ω , during spin-down as a pulsar, will decrease the difference $\varepsilon_\Omega - \varepsilon_0$; the present reference oblateness is $\varepsilon_0 \approx \varepsilon_0(0) \sim 10^{-6}$. For large $\theta_m \sim 10^{-1}$, one may consider the possibility that ε_0 grows by solidification of accreted material in a way that does not follow the evolution of ε_Ω . However, the magnetic field that channels the accreted material plays a negligible part in the equilibrium energy balance, on the star's surface in comparison with rotation; $(B^2/8\pi)(4\pi R^3/3I_0\Omega^2)$ is $\sim 4 \times 10^{-6}$, for $B = 10^{12}$ G and $P = 1$ s, and $\sim 10^{-19}$, for $B = 10^8$ G and $P = 1.5$ ms. Furthermore, although the total amount of matter accreted is $\sim 0.1 M_\odot$, it is unlikely that this can adhere, in a metastable way, to the magnetic-field-accretion geometry and solidify to change ε_0 on time-scales as long as the accretion time of $\sim 10^8$ yr; the temperature of the accreted matter is in the X-ray range through this era, so

Table 1 Values of ε_{eff} and ε_{Ω} for the millisecond and binary pulsars

Pulsar	1937+21	1953+29	1913+16	0820+02	0655+64	'Ordinary'
$P(\text{ms})$	1.6	6.1	59	865	196	200
$\dot{P}(10^{-19} \text{ s s}^{-1})$	1.05*	7.8†	86	10 ³	<10‡	10 ⁵
$\varepsilon_{\text{eff}}(I_{45}^{-1/2})$	3.8×10^{-9}	8×10^{-8}	8×10^{-6}	1.5×10^{-3}	$<1.6 \times 10^{-5}$	1.7×10^{-3}
$\varepsilon_{\Omega}(I_{45}/A_{53})^{\S}$	4.1×10^{-2}	2.7×10^{-3}	2.8×10^{-5}	1.3×10^{-7}	2.5×10^{-6}	2.5×10^{-6}

The last column lists ε_{eff} and ε_{Ω} for P and $\dot{P}$ values typical of an ordinary pulsar.

* Ref. 3.

† Scaling as $P^{4/3}$ from the observed value for PSR1937+21. The present observational upper limit is $\dot{P} < 4.2 \times 10^{-17}$.

‡ Present upper limit (A. G. Lyne, personal communication).

§ A is the coefficient in the additional gravitational potential energy, $A\varepsilon^2$, of the star due to an oblateness ε with typical values $\sim 10^{53}$ erg (ref. 16).

diffusion and plastic flow timescales are likely to be much shorter than 10^8 yr.

(3) The angle θ_0 between $\hat{n}_0$ and $\hat{n}_{\Omega}$. This can also change through quakes. Pines and Shaham²⁰ have shown that the critical value of θ_0 for such a ' θ -quake' is reached when $3\varepsilon_{\Omega}\varepsilon_0\theta_0^2 = \theta_m^2$. The different possibilities are: (i) for $\theta_m \sim 10^{-4}$ and $\varepsilon_0 \approx \varepsilon_{\Omega} \sim 10^{-1}$, the critical value of θ_0 is $\sim 10^{-3}$. Thus, a crust with a small critical angle cannot sustain any misalignment between $\hat{n}_0$ and $\hat{n}_{\Omega}$ greater than $\sim 10^{-3}$ rad. $\theta_0 = 0$ at initial solidification and cannot exceed $\sim 10^{-3}$ through the star's history. (ii) For $\theta_m \sim 10^{-1}$, $\varepsilon_{\Omega} \approx 10^{-1}$ and $\varepsilon_0 \approx 10^{-6}$, no bound is imposed on θ_0 . Starting at $\theta_0 = 0$ at solidification, θ_0 could in principle grow arbitrarily, without inducing a ' θ -quake' in such a strong crust; but one needs an evolutionary reason for a secular increase of θ_0 and the only candidate, solidification of accreted material persisting through the 10^8 -yr accretion phase, is ruled out by the considerations given above in connection with the related question of the evolution of ε_0 for a $\theta_m \sim 0.1$ crust.

(4) The effect of pinned superfluidity. The presence of a pinned superfluid will not alter the situation. Shaham²¹ has investigated the effect of the pinned superfluid on the rotational dynamics of a neutron star. Pinning leads to an angular momentum $\vec{L}_f$ that is constant in the rotating frame of the star. Shaham shows that the precession frequency in the rotating frame is of the order of L_f/I_0 . We find

$$\frac{L_f}{I_0} \approx \frac{I_p}{I_0} (\Omega + \omega_{\text{cr}}) \approx \frac{I_p}{I_0} \Omega \approx 10^{-2} \Omega \quad (12)$$

where $I_p \approx 10^{-2} I_0$ is the moment of inertia of the pinned superfluid and $\omega_{\text{cr}} \approx 1 \text{ rad s}^{-1}$ is a critical frequency for unpinning^{22,23}. The corresponding frequency in the non-rotating frame is given by

$$\Omega_{\text{pr}} = \Omega(1 + O(\frac{L_f}{I_0\Omega})) + \frac{3}{2} b\varepsilon_0 \cos^2 \theta_0 = \Omega(1 + O(10^{-2})) \approx \Omega \quad (13)$$

For the values of L_f given by neutron star pinning parameters, the gravitational radiation emitted by the neutron star deviates little from that in the absence of pinning. As Ω_s , the rotation rate of the pinned superfluid, exceeds Ω by at most the critical frequency, ω_{cr} , that is, $|\Omega_s - \Omega| < \omega_{\text{cr}} \approx 1 \text{ rad s}^{-1}$, the angle ϕ between $\vec{L}_f$ and $\vec{\Omega}$ will be small, that is, $\phi < \omega_{\text{cr}}/\Omega \sim 10^{-3}$. The angle $\theta_0 - \theta_L$ between $\vec{\Omega}$ and $\vec{L}$ is also small. Using $\vec{L} = \vec{I} \cdot \vec{\Omega} + \vec{L}_f$ we find $\theta_0 - \theta_L \approx (I_p/I_0)\phi < (I_p/I_0)(\omega_{\text{cr}}/\Omega) \sim 10^{-5}$. The deviations of $\vec{\Omega}$ and $\vec{L}_f$ from the direction of $\vec{L}$ are very limited. A comparison with our calculation for gravitational radiation in the absence of pinning shows that the only effect on $M(t)$ is to replace $O(b\varepsilon_0)$ expressions for the angle $(\theta_L - \theta_0)$ with another negligible quantity, as $\theta_L - \theta_0$ is now $(I_p/I_0)(\omega_{\text{cr}}/\Omega) \sim 10^{-5}$. The lowest-order results for the gravitational radiation output are not affected, nor are our evaluations of the effective triaxiality under different assumptions for the strength of the crust solid.

(5) The expected value of ε_{eff} . We thus conclude that the effective triaxiality, ε_{eff} , in equation (11) is: (i) $\varepsilon_{\text{eff}} \approx \frac{3}{8} b\varepsilon_0 \theta_0 \approx \frac{3}{8} \times 10^{-5} \times 10^{-1} \times 10^{-3} \approx 4 \times 10^{-10}$, if $\theta_m \sim 10^{-4}$; (ii) $\varepsilon_{\text{eff}} \approx b\varepsilon_0 \approx 10^{-5} \times 10^{-6} \approx 10^{-11}$, if $\theta_m \sim 10^{-1}$.

(6) Intrinsic triaxiality. Intrinsic triaxiality of the solid reference figure adds a term $\eta(\hat{l}_0\hat{l}_0 - \hat{m}_0\hat{m}_0)$ to $\vec{I}$ in equation (3), where η is the 'reference triaxiality', and $\hat{l}_0, \hat{m}_0, \hat{n}_0$ form an orthogonal frame. It follows that ε_{eff} acquires a term of the order of $b\eta$, and that $\eta \leq \theta_m$. Thus, $b\eta \leq 10^{-5} \times 10^{-4} \approx 10^{-9}$, if $\theta_m \sim 10^{-4}$. In the 'no quake' case, $\theta_m \sim 10^{-1}$, η retains its initial value, $\eta = \eta(0) \approx \varepsilon_0(0) \sim 10^{-6}$, and one finds $b\eta \approx 10^{-5} \times 10^{-6} \approx 10^{-11}$, if $\theta_m \sim 10^{-1}$. Both limits are less than the observational limit ε_{max} .

In conclusion, our results in parts (5) and (6) above show that gravitational radiation poses no problem for the observed $\dot{P}$ values of millisecond pulsars. A consideration of damping of the solid crust's precession through its coupling to the superfluid neutron star core strengthens this conclusion. The damping time τ_w is^{24,25}

$$\tau_w \approx \frac{\Omega}{\Omega'_{\text{pr}}} \tau \approx (\frac{3}{2} b\varepsilon_0)^{-1} \tau \quad (14)$$

where Ω'_{pr} is the precession frequency in the body frame and τ is the crust-core coupling time. A recent investigation²⁶ of the interaction of quantized vortices in the core neutron superfluid with electrons and protons yields a coupling time $\tau_c \approx 20P(\text{s})$. The dynamical coupling time τ for the neutron superfluid is then $\tau(\text{s}) \approx \tau_c/x \approx 400P(\text{s})$, where x , the ratio of the number of protons to that of neutrons, is typically $\sim 5\%$. From equation (14) and the above value of τ , we obtain a damping time $\tau_w(\text{yr}) \leq 1,300 \text{ yr}$ for PSR1937+21, using $b\varepsilon_0 \sim 10^{-11}$, whereas with pinning, $\Omega/\Omega'_{\text{pr}} \sim 100$ and $\tau_w \approx 1 \text{ min}$.

We thank Fred Lamb and Naoki Iwamoto for bringing this problem to our attention, Mal Ruderman and Jacob Shaham for useful discussions and Jim Applegate and Roger Blandford for pointing out an error in our initial calculations. This research was supported by research grants NSF PHY80-25605 and NASA NSG-7653.

Received 3 December 1984; accepted 15 January 1985.

- Ashworth, M., Lyne, A. G. & Smith, F. G. *Nature* **301**, 313-314 (1983).
- Backer, D. C., Kulkarni, R. S. & Taylor, J. H. *Nature* **301**, 314-315 (1983).
- Backer, D. C. in *Proc. Workshop on Millisecond Pulsars* (eds Reynolds, S. & Stinebring, D.) 3-8 (NRAO, Greenbank, West Virginia, 1984).
- Backer, D. C., Kulkarni, R. S., Heiles, C., Davis, M. M. & Goss, W. M. *Nature* **300**, 615-618 (1982).
- Boriakoff, V., Buccheri, R., Fauci, F., Turner, K. & Davis, M. in *Proc. Workshop on Millisecond Pulsars* (eds Reynolds, S. & Stinebring, D.) 24-29 (NRAO, Greenbank, West Virginia, 1984).
- Boriakoff, V., Buccheri, R. & Fauci, F. *Nature* **304**, 417-419 (1983).
- Alpar, M. A., Cheng, A. F., Ruderman, M. A. & Shaham, J. *Nature* **300**, 728-730 (1982).
- Radhakrishnan, V. & Srinivasan, G. *Curr. Sci.* **51**, 1096-1099 (1982).
- Fabian, A. C., Pringle, J. E., Verbunt, F. & Wade, K. A. *Nature* **301**, 222-223 (1983).
- Pines, D. & Shaham, J. *Phys. Earth planet. Inter.* **6**, 103-115 (1972).
- Wagoner, R. *Astrophys. J.* **278**, 345-348 (1984).
- Friedman, J. L. *Phys. Rev. Lett.* **51**, 11-14 (1983).
- Landau, L. D. & Lifshitz, E. M. *Mechanics* Ch. 6 (Pergamon, Oxford, 1976).
- Weinberg, S. *Gravitation and Cosmology* Ch. 10 (Wiley, New York, 1972).
- Shapiro, S. L. & Teukolsky, S. A. *Black Holes, White Dwarfs and Neutron Stars* Ch. 16 (Wiley-Interscience, New York, 1983).
- Pandharipande, V. R., Pines, D. & Smith, R. A. *Astrophys. J.* **208**, 550-566 (1976).
- Ruderman, M. A. *Nature phys. Sci.* **223**, 597-598 (1969).
- Baym, G. & Pines, D. *Ann. Phys.* **66**, 816-835 (1971).
- Smolouchowski, R. *Phys. Rev. Lett.* **24**, 923-925 (1970).
- Pines, D. & Shaham, J. *Nature phys. Sci.* **235**, 43-49 (1972).
- Shaham, J. *Astrophys. J.* **214**, 251-260 (1977).
- Alpar, M. A., Anderson, P. W., Pines, D. & Shaham, J. *Astrophys. J.* **276**, 325-334 (1984).
- Pines, D. & Alpar, M. A. *Proc. Workshop on Millisecond Pulsars* (eds Reynolds, S. & Stinebring, D.) 161-169 (NRAO, Greenbank, 1984).
- Bondi, H. & Gold, T. *Mon. Not. R. astr. Soc.* **115**, 41-46 (1955).
- Pines, D. & Shaham, J. *Nature* **248**, 483-486 (1974).
- Alpar, M. A., Langer, S. A. & Sauls, J. A. *Astrophys. J.* **282**, 533-541 (1984).

Hubble's constant and exploding carbon-oxygen white dwarf models for Type I supernovae

W. David Arnett*, David Branch† & J. Craig Wheeler‡

* Enrico Fermi Institute, University of Chicago, Chicago, Illinois 60637, USA

† Department of Physics and Astronomy, University of Oklahoma, Norman, Oklahoma 73019, USA

‡ Department of Astronomy, University of Texas, Austin, Texas 78712, USA

The immediate progenitor of a Type I supernova (SN I) is thought to be a mass-accreting carbon-oxygen (C-O) white dwarf in a binary system. When the mass of the white dwarf approaches the Chandrasekhar mass ($1.4 M_{\odot}$) the C-O nuclear fuel ignites, part of the star is incinerated to radioactive ^{56}Ni , and the thermonuclear energy completely disrupts the star. The optical luminosity results from the trapping and thermalization of the γ rays and positrons emitted by the decay of ^{56}Ni through ^{56}Co to stable ^{56}Fe . The amount of ^{56}Ni synthesized, M_{Ni} , and the corresponding peak luminosity, L_{max} , can be used with the observed Hubble diagram for SN I to determine the value of Hubble's constant, H_0 . We argue here that if this model is correct, M_{Ni} is in the range 0.4 – $1.4 M_{\odot}$, the best estimate being $0.6 M_{\odot}$, and that H_0 is in the range 39 – $73 \text{ km s}^{-1} \text{ Mpc}^{-1}$ with a best estimate of $59 \text{ km s}^{-1} \text{ Mpc}^{-1}$. This line of reasoning does not require knowledge of the temperature of the supernova and, therefore, is not subject to the uncertainties associated with attempts to determine supernova luminosities and distances by the Baade method¹. It relies on the physical correctness of the model, which is subject to independent tests.

For SN I, analytical solutions² for the light curves predict that at the time of maximum bolometric luminosity, L_{max} is equal to the instantaneous decay luminosity of the ^{56}Ni and ^{56}Co independent of the opacity and the precise form of the deposition function. Numerical calculations confirm the insensitivity of the peak bolometric luminosity to opacity³ and show that the radiated power is equal to the rate of radioactive deposition at bolometric maximum to within 15%. The time between explosion and maximum luminosity in the B-band is observed to be 15 ± 2 days (ref. 4), which implies that maximum bolometric luminosity occurs at 17 ± 2 days (ref. 3). It follows that $L_{\text{max}} = 2.2 \pm 0.2 \times 10^{43} M_{\text{Ni}} \text{ erg s}^{-1} M_{\odot}^{-1}$. This is a significant simplification; at times away from maximum luminosity, the luminosity is affected by the opacity of the matter and hence its composition, thus introducing additional uncertainty.

For an exploding white dwarf, a rigorous and extreme upper limit on M_{Ni} is $1.4 M_{\odot}$. Optical spectroscopy of extragalactic SN I⁵ and X-ray spectroscopy of remnants of SN I in our Galaxy (C. Sarazin, personal communication) show that the ejected matter contains synthesized elements such as silicon and sulphur, and unburned carbon (presupposing the model) and oxygen in addition to iron-peak elements so the entire $1.4 M_{\odot}$ cannot be ^{56}Ni .

A lower limit on M_{Ni} follows from the observed kinetic energy and velocities. To an excellent approximation the kinetic energy of ejected matter is the difference between the thermonuclear energy released and the net binding energy of the white dwarf. Nuclear conversion of C-O to ^{56}Ni produces $1.4 \times 10^{51} \text{ erg } M_{\odot}^{-1}$. Detailed hydrodynamic calculations^{6,7} for exploding white dwarfs show, however, that less than half of the material actually becomes ^{56}Ni . Some of the energy release is associated with the production of other elements. Calculations, which treat the nonradial flow in a crude way but the nucleosynthesis in detail^{8,9}, give a similar result; $3.1 \times 10^{51} \text{ erg}$ of nuclear energy is released per solar mass of synthesized ^{56}Ni . The net binding energy is $0.5 \times 10^{51} \text{ erg}$, so $E_{\text{K}}/2/10^{51} = 3.1 M_{\text{Ni}} - 0.5$. Note that E_{K} depends strongly on M_{Ni} for $M_{\text{Ni}} < 0.4 M_{\odot}$, and goes to zero as M_{Ni} approaches $0.16 M_{\odot}$. Numerical simulations³ have shown that

at least $0.4 M_{\odot}$ of ^{56}Ni (corresponding to $0.74 \times 10^{51} \text{ ergs}$ of nuclear energy) must be synthesized to account for velocities at the photosphere, inferred from optical spectroscopy⁵, of $10,000$ – $12,000 \text{ km s}^{-1}$ during the first few weeks after maximum light. We regard $M_{\text{Ni}} > 0.4 M_{\odot}$ as a conservative lower limit because, in the context of the model, it implies that, although another $\approx 0.4 M_{\odot}$ is burned to other species, $0.6 M_{\odot}$ of C-O is not burned at all. Smaller amounts of C-O in the ejecta are indicated by both the optical⁵ and X-ray spectroscopy (C. Sarazin, personal communication). Taking all available evidence, we consider the best current estimate for the nickel produced to be $M_{\text{Ni}} = 0.6 M_{\odot}$ ($1.86 \times 10^{51} \text{ ergs}$ of nuclear energy). Thus, our best estimate of L_{max} is $1.3 \times 10^{43} \text{ erg s}^{-1}$, with limits of 3.05×10^{43} ($1.4 M_{\odot} \text{ Ni}$) and $0.87 \times 10^{43} \text{ erg s}^{-1}$ ($0.4 M_{\odot} \text{ Ni}$).

The transformation from bolometric luminosity L_{max} to absolute blue magnitude M_B , required for comparison with observed SN I, is straightforward^{3,10}. At the time of maximum luminosity, the observed flux distribution for $\lambda > 0.4 \mu\text{m}$ resembles that of a $20,000 \text{ K}$ blackbody, while only a small fraction of the flux is emitted at $\lambda < 0.4 \mu\text{m}$ (ref. 5). When the model luminosity is distributed in this way, we find $M_B = 19.23 - 2.5 \log L_{\text{max}}/10^{43}$. Note that the colour temperature enters only as a convenient parameter for distributing the model luminosity over wavelength, and that the relation between L_{max} and M_B depends only weakly on its value. An understanding of the atmospheric processes which determine the flux distribution is not required for our present purpose. We simply use the observed distribution. The best estimate of M_B is -19.5 , with limits of -20.4 and -19.1 .

The predicted values of M_B are related to H_0 through the observed dependence of the apparent magnitudes of SN I on the redshifts of their parent galaxies. To bypass the issues of interstellar extinction in the parent galaxies and departures from ideal Hubble flow in the local Virgo supercluster we restrict our attention to SN I in elliptical and lenticular galaxies having recession velocities $> 3,000 \text{ km s}^{-1}$. There are six such SN I (including three in the Coma cluster) having well-determined apparent magnitudes at peak. After allowing for interstellar extinction in our Galaxy¹¹, using $M_B = M_{\text{pg}} + 0.3$ (ref. 12) when necessary, and allowing for a difference of 0.1 magnitude between the apparent blue magnitude at 15 (optical peak) and 17 days (bolometric peak) after the explosion we find $M_B = -18.4 \pm 0.2 + 5 \log(H_0/100)$, in good agreement with calibrations done previously^{12,13}. The predicted values of M_B then correspond to a best estimate of $H_0 = 59 \text{ km s}^{-1} \text{ Mpc}^{-1}$, a rigorous lower limit of $39 \text{ km s}^{-1} \text{ Mpc}^{-1}$, and a conservative upper limit of $73 \text{ km s}^{-1} \text{ Mpc}^{-1}$. The uncertainties in the time of bolometric maximum and in the absolute magnitude calibration give an r.m.s. uncertainty for each of these values of $\approx 10\%$. This is small compared with the uncertainty associated with the ejected nickel mass.

The arguments expressed above can be summarized in the relationship $H_0 = 46 \pm 4 M_{\text{Ni}}^{-1/2} \text{ km s}^{-1} \text{ Mpc}^{-1}$. We have argued that values of H_0 in excess of about $73 \text{ km s}^{-1} \text{ Mpc}^{-1}$ would require $M_{\text{Ni}} < 0.4 M_{\odot}$, and that the corresponding kinetic energy and velocities would be too small. A high value of H_0 could be reconciled with exploding white dwarfs as SN I if only a portion of the matter is ejected, with the remainder forming a neutron star. Kinetic energy and nickel mass would become uncoupled because some kinetic energy would come from the binding energy of the neutron star. Studies of galactic remnants of SN I indicate, however, that there is no evidence that neutron stars are left behind^{14,15}.

The discussion above has rested on the tacit assumption that all SN I have identical properties. Observationally, this is a tenable proposition, except for a few supernovae (SN 1885a, 1954a, 1962l, 1964l, 1980i, 1983n) which have shown definite spectroscopic and/or photometric peculiarities, and theoretically it is attractive because the ignition of carbon necessarily occurs near the Chandrasekhar mass. There is some evidence, however, for a correlation between peak absolute magnitude and the rate of decline of the initial post-peak light curve^{16–18}.

If the peak luminosities of normal SN I do cover a finite range, the values of H_0 quoted here are likely to be too high, because, owing to observational selection effects, the SN I beyond the local supercluster will tend to be more luminous than the much nearer SN I, such as 1972e in NGC5253 and 1981b in NGC4536, whose characteristics are used to estimate the nickel mass.

These results are entirely independent of methods for determining distance to Type II supernovae, which involve an understanding of the atmosphere and spectral line-forming regions. In addition, although model dependent, this method is independent of the traditional 'distance ladder' approach.

We thank the Aspen Center for Physics for hospitality and Gary Steigman, Peter Sutherland and Stan Woosley for comments and discussion. This research was supported in part by NSF grants AST 8316815 (W.D.A.), AST 8218625 (D.B.) and AST 8201210 (J.C.W.).

Received 10 October 1984; accepted 14 January 1985.

1. Wagoner, R. V. *Astrophys. J.* **250**, L65-L69 (1981).
2. Arnett, W. D. *Astrophys. J.* **253**, 785-797 (1982).
3. Sutherland, P. G. and Wheeler, J. C. *Astrophys. J.* **280**, 282-297 (1984).
4. Barbon, R., Ciatti, F. & Rosino, L. *Astr. Astrophys.* **25**, 241-248 (1973).
5. Branch, D. *et al. Astrophys. J.* **270**, 123-139 (1983).
6. Müller, E. & Arnett, W. D. *Astrophys. J. Lett.* **261**, L109-L115 (1982).
7. Müller, E. & Arnett, W. D. in *Nucleosynthesis: Problems and Challenges* (eds Arnett, W. D. & Truran, J. W.) (University of Chicago, in the press).
8. Nomoto, K., Thielmann, F.-K. & Yokoi, K. *Astrophys. J.* **268**, 644-658 (1984).
9. Woosley, S. E., Axelrod, T. S. & Weaver, T. A. in *Stellar Nucleosynthesis* (eds Chiosi, C. & Renzini, A.) (Reidel, Dordrecht, 1984).
10. Schurmann, S. R. *Astrophys. J.* **267**, 779-794 (1983).
11. Burstein, D. & Heiles, C. *Astrophys. J.* **225**, 40-55 (1978).
12. Branch, D. & Bettis, C. *Astr. J.* **83**, 224-227 (1978).
13. Tammann, G. A. in *Supernovae: A Survey of Current Research* (eds Rees, M. J. & Stoneham, R. J.) 371-403 (Reidel, Dordrecht, 1982).
14. Helfand, D. J. in *Type I Supernovae* (ed. Wheeler, J. C.) 20-24 (University of Texas, Austin, 1980).
15. Nomoto, K. & Tsuruta, S. *Astrophys. J. Lett.* **250**, L19-L23 (1981).
16. Rust, B. W. thesis, Univ. Illinois, Urbana (1974).
17. Pskovskii, Y. P. *Soviet Astr.-A. J.* **21**, 675-682 (1977).
18. Branch, D. *Astrophys. J.* **258**, 35 (1982).

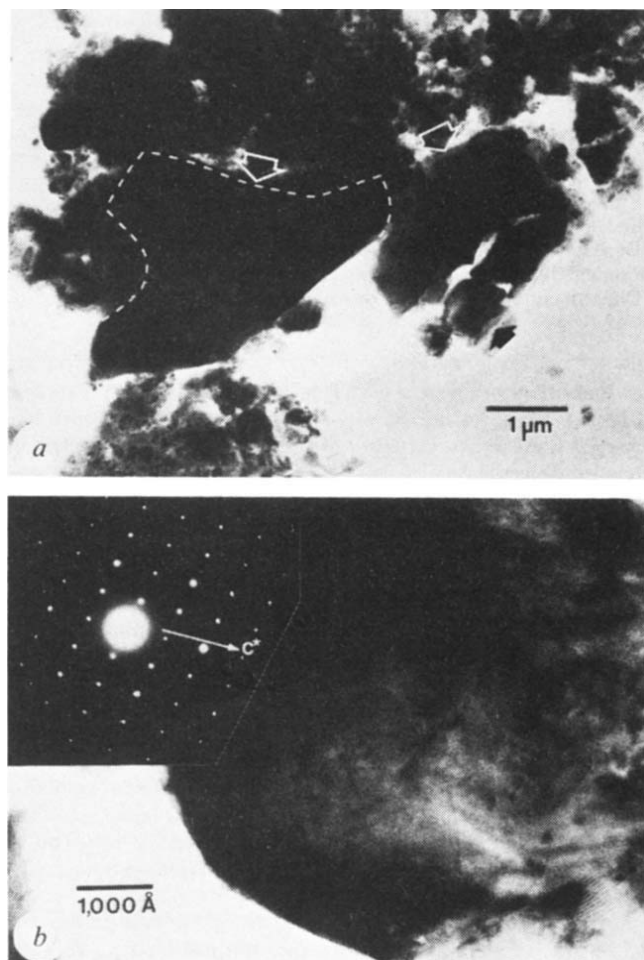


Fig. 1 *a*, Fassite grains (indicated by arrows) in one of the Skywalker aggregates; surrounding them are clusters of the smectite/mica and pyrrhotite grains. The clustered grains on the right are apparently broken fragments of a single grain, $\sim 2.5 \mu\text{m}$ in diameter. The grain on the left is the largest single crystal found in Skywalker. *b*, Magnified image of the fassite grain indicated by a small arrow in *a*, showing solar-flare tracks. The SAED pattern in the inset, obtained from the same grain, corresponds to [101] of clinopyroxene.

Hydrated interplanetary dust particle linked with carbonaceous chondrites?

Kazushige Tomeoka & Peter R. Buseck

Departments of Geology and Chemistry, Arizona State University, Tempe, Arizona 85287, USA

Recent mineralogical and geochemical studies of interplanetary dust particles (IDPs) provide convincing evidence that they contain materials that formed in the early solar nebula. Some chondritic IDPs contain hydrated phases and may be related to CI and CM carbonaceous chondrites. However, detailed mineralogical data of the hydrated IDPs are extremely limited. We report here the results of transmission electron microscope (TEM) observations of a hydrated IDP containing Fe-, Mg-rich smectite or mica as a major phase. The sheet silicate appears to have formed by alteration of anhydrous silicates. Fassite, a Ca, Al clinopyroxene, also occurs in this particle, and one of the crystals exhibits solar-flare tracks, clearly indicating that it is extraterrestrial. Fassite is a major constituent of the Ca-, Al-rich refractory inclusions (CAIs) found in the carbonaceous chondrites. Its presence in this particle, therefore, suggests that there may be a link between hydrated IDPs and carbonaceous chondrites in the early history of the Solar System.

The hydrated particle studied here, 'Skywalker' (NASA no. r21-M3-5A), was collected from the upper stratosphere by NASA U-2 aircraft. Sandford *et al.*¹ showed that its infrared spectrum contains absorption bands at $\sim 3,400 \text{ cm}^{-1}$ and $\sim 1,600 \text{ cm}^{-1}$, which they attributed to the presence of water. A large deuterium enrichment, measured with the ion probe², provides strong evidence that Skywalker contains a unique isotope record, and this was probably established in or before the early Solar System³. Thus, Skywalker is of great interest,

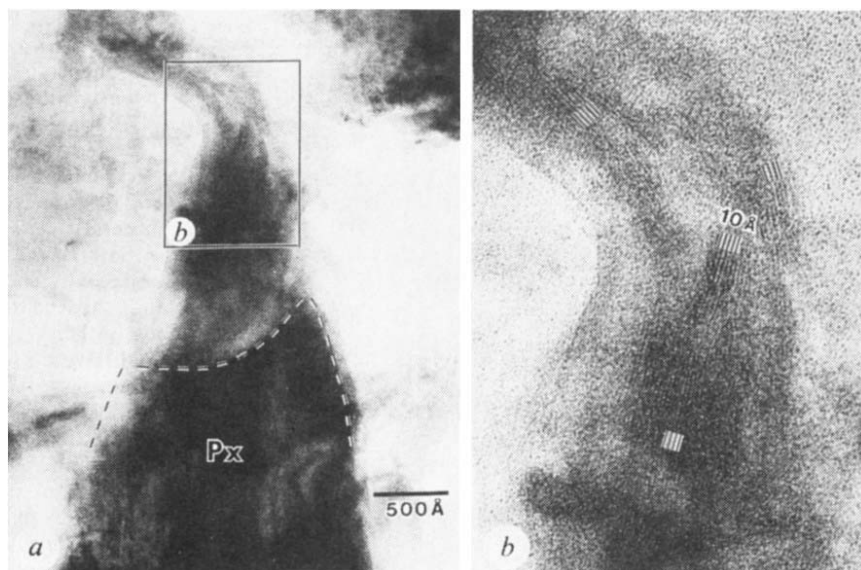
particularly regarding its relations to the carbonaceous chondrites.

TEM imaging and chemical analysis by energy-dispersive X-ray spectroscopy (EDS) have been used to study the microstructures and compositions of the constituent minerals of Skywalker. Structural identifications were made by selected-area electron diffraction (SAED), convergent-beam electron diffraction and high-resolution imaging. The analyses of individual grains were performed using a finely focused beam ($\sim 800 \text{ Å}$ in diameter). Quantitative data were derived for silicate minerals using thin-film X-ray microanalysis⁴. Details of the experimental procedure and reference standards for the quantitative analyses are given elsewhere⁵.

A portion of Skywalker was placed on a carbon film, then mounted on a TEM grid. The sample on the grid consists of six aggregates, ranging from 4 to 7 μm in size, and numerous finer grains. EDS spectra, obtained using a broad beam, show that the particle falls into the chondritic subset of Brownlee⁶. Based on its infrared spectrum, Sandford *et al.*¹ placed Skywalker in the small group of hydrated IDPs.

The pyroxene occurs as a cluster of five grains that range up to 4.5 μm in diameter; four are apparently broken fragments of a single crystal (Fig. 1). Their SAED patterns correspond to clinopyroxene. The grains contain major amounts of Mg, Al

Fig. 2 *a*, A low-magnification TEM image of an enstatite grain. Above the dashed line is the phyllosilicate. *b*, A high-resolution TEM image of the phyllosilicate from the boxed area in *a*, displaying the curved fringes with ~ 10 -Å spacing.



and Ca and minor amounts of Ti, Cr and Fe (Table 1). Using the ratios given in Table 1, the proposed chemical formula is $[\text{Ca}_{0.7}\text{Mg}_{0.8}\text{Al}_{0.4}](\text{Si}_{1.8}\text{Al}_{0.2})\text{O}_6$, which is consistent with its designation as fassaite⁷.

Fassaite occurs in both terrestrial and meteoritic samples. It is of special interest in meteorites because it is a major constituent of the primitive and refractory CAIs that are prominent in the CV carbonaceous chondrites^{8,9} and that also occur in the CM^{10,11} and ordinary chondrites^{12,13}. Fassaite in CAIs shows a wide range of Al content (9–22 wt% Al_2O_3) and Ti content (2–18 wt% TiO_2), but it is commonly enriched in both these elements and depleted in Fe relative to fassaite in terrestrial samples^{8,9}. The fassaite in Skywalker is aluminous, low in Ti and Fe (Table 1). The high Al and low Fe contents distinguish Skywalker fassaite from terrestrial fassaite⁷. Furthermore, the limited compositional similarity between the fassaite in the IDP and the carbonaceous chondrites raises at least the possibility that there is a genetic relationship between the two occurrences.

Fossil nuclear tracks occur in one of the fassaite grains (Fig. 1*b*). Such tracks in IDPs were first described from olivines and pyroxenes in ordinary chondritic IDPs by Bradley *et al.*¹⁴. The

tracks probably resulted from IDP exposure to solar cosmic rays before capture by the Earth¹⁵. Laboratory experiments show that nuclear tracks in silicates can be annealed by pulse (2 s) heating to 600 °C (ref. 15). Thus, the tracks provide evidence both that fassaite in Skywalker is extraterrestrial and that it has not been severely heated since track implantation, even during atmospheric entry.

Both Skywalker and Low-Ca⁵, which is the only other hydrated IDP that has been studied by TEM, contain a mineral that has a distinctly layered appearance in TEM images. It contains Si, Fe, Mg, Al and has an interlayer spacing of 10–12 Å; average cation/silicon ratios for the mineral in Skywalker are 0.30 (Mg/Si), 0.28 (Fe/Si) and 0.08 (Al/Si), and for the mineral in Low-Ca they are 0.49 (Mg/Si), 0.26 (Fe/Si) and 0.09 (Al/Si). Based on these criteria, plus the infrared evidence for hydrated phases, we⁵ have identified it in Low-Ca as an Fe-, Mg-rich smectite or mica, which we hereafter call smectite/mica. The same identification also holds for Skywalker.

Skywalker also contains small amounts of an Fe-rich phyllosilicate; average cation/silicon ratios are 0.21 (Mg/Si), 0.79 (Fe/Si) and 0.09 (Al/Si). Both smectite/mica and Fe-rich phyllosilicate occur as aggregates of poorly crystalline, fine fibres similar to those illustrated in ref. 5. As in Low-Ca, smectite/mica occurs in close association with Fe-Ni sulphides, mostly rounded grains ($\sim 1,000$ Å) of nickeliferous pyrrhotite, whereas the Fe-rich phyllosilicate is rarely associated with sulphides. The Fe-rich phyllosilicate exhibits weak powder electron-diffraction lines at ~ 4.6 , ~ 2.6 and ~ 1.5 Å. Its poor crystallinity hindered further structural characterization. Smectite/mica in Skywalker is commonly more Fe-rich and Mg-poor and shows a wider variation in these elements than that in Low-Ca. This variation probably results from partial mixing with the Fe-rich phyllosilicate.

Platelets of enstatite pyroxene and forsterite olivine, containing up to a few atomic per cent of Fe and ranging in diameter from 0.1 to 3 μm , are common in Skywalker, in marked contrast to Low-Ca, where olivine is rare and no pyroxene was found⁵. Olivine platelets commonly display strong strain-field contrast and complex patterns of Moiré fringes. Pyroxene platelets typically show a disordered stacking of clino- and ortho-enstatite^{16,17} (Fig. 3). Similar platelets of olivine and pyroxene are also common in anhydrous IDPs^{18,19}. Bradley *et al.*¹⁹ suggested that such pyroxene platelets are probably primary vapour-phase condensates that could have formed in the primitive solar nebula.

Figure 2 shows a grain consisting of Mg-Fe pyroxene at one end and phyllosilicate at the other. The high-resolution TEM image (Fig. 2*b*) displays curved fringes characteristic of phyllosilicate and having a spacing of ~ 10 Å. EDS analysis indicates

Table 1 Thin-film, X-ray microanalyses (oxide weight percentages and cation ratios per six oxygens) of fassaite from the Skywalker IDP and microprobe analyses of fassaites from meteoritic CAIs and eclogite

	Skywalker*	Allende ⁹	Murchison ¹¹	Eclogite ³³ (terrestrial)
Oxide (wt%)				
MgO	14.9 (1.2)	11.51	12.34	15.13
Al_2O_3	14.5 (0.8)	15.14	15.91	7.41
SiO ₂	49.6 (1.9)	42.90	42.41	50.85
CaO	18.7 (1.2)	26.02	24.34	17.37
TiO ₂	0.6 (0.1)	6.01	3.53	0.78
Cr ₂ O ₃	0.5 (0.1)	—	—	—
FeO	1.2 (0.7)	0.03	—	5.99
Total	100.0	101.58	98.53	97.53†
No. of tetrahedral cations on the basis of six O				
Si	1.75	1.54	1.56	1.84
Al	0.25	0.46	0.44	0.16
Total	2.00	2.00	2.00	2.00
No. of other cations				
Al	0.35	0.18	0.26	0.16
Cr	0.01	—	—	—
Ti	0.02	0.16	0.10	0.02
Mg	0.79	0.62	0.68	0.82
Fe	0.04	0.00	—	0.18
Ca	0.71	1.00	0.96	0.67
Total	1.92	1.96	1.99	1.85

* Average of analyses from 12 different points. Values in parentheses are standard deviations. Original data were obtained in relative ratios, then normalized to 100%.

† Na, K and Mn are excluded.

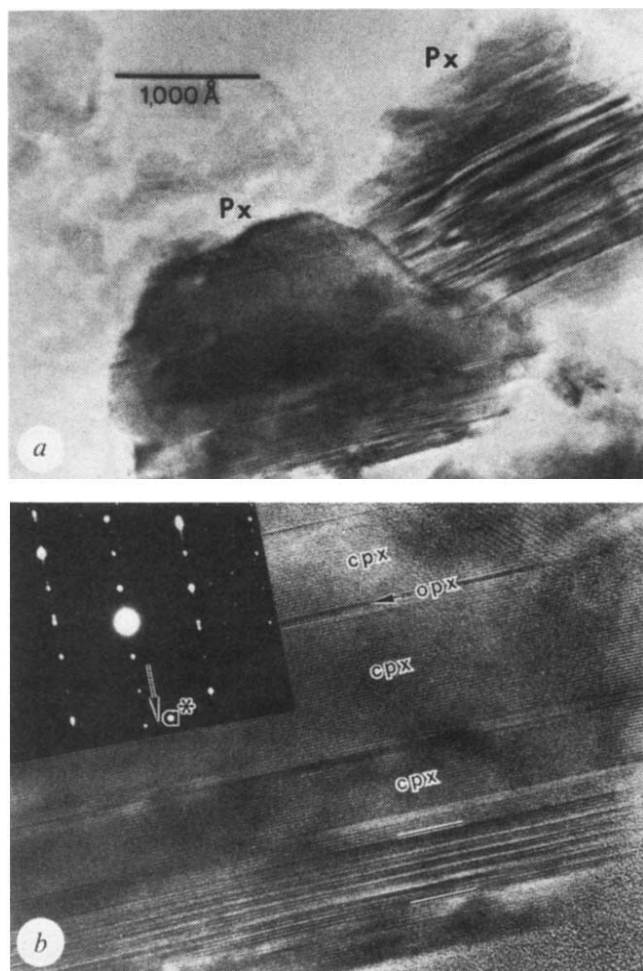


Fig. 3 *a*, Enstatite platelets. *b*, A high-resolution TEM image of part of the pyroxene grain at the bottom of *a* and its SAED pattern (inset). The image shows regions of clinopyroxene (cpx), orthopyroxene (opx) and stacking disorder of cpx and opx (between lines); cpx and opx have fringe spacings of 9 and 18 Å. Streaking of reflections along a^* in the diffraction pattern results from stacking disorder of cpx and opx.

that the phyllosilicate is much richer in Fe than the pyroxene; Al was not detected from either the pyroxene or the phyllosilicate. Although the actual boundary between the two minerals is poorly defined, the external edge is clearly continuous around both minerals, and the pyroxene and the phyllosilicate are certainly parts of the same grain. Many small grains of smectite/mica also occur along the edges of other pyroxene crystals. By analogy with images obtained of terrestrial^{20,21}, meteoritic²² and synthetic samples²³, we believe that the phyllosilicate was produced by alteration of the pyroxene. It is, therefore, reasonable to assume the past occurrence of aqueous alteration in the history of this IDP, although it is not possible to say whether liquid or vapour was responsible for the alteration.

An unidentified mineral containing major Fe, Mg and minor Ca, Mn and Si is widespread and is commonly associated with smectite/mica; it occurs in rounded grains, 500 Å to 1 µm in diameter. A similar phase was also found in Low-Ca⁵, but it has not been reported from either other IDPs or meteorites and so may be unique to the hydrated IDPs.

Fassaite has not been reported previously from IDPs; its discovery provides important information regarding the origin of the hydrated IDPs. The bulk compositions of the chondritic IDPs as well as their isotope compositions and mineralogy suggest a strong similarity between IDPs and carbonaceous chondrites. If fassaite in Skywalker formed by processes related to the formation of CAIs, then its presence provides evidence

suggesting that the hydrated IDPs contain primitive materials that formed in the early solar nebula. The hydrated IDPs, like carbonaceous chondrites, presumably have not experienced extensive high-temperature metamorphism since their accretion.

Although there are definite mineralogical similarities between hydrated IDPs and carbonaceous chondrites, our TEM studies also reveal important differences. The phyllosilicates in the CI and CM meteorites are mostly serpentine- or sepioclaurite-type minerals²⁴⁻²⁹ rather than the Fe- and Mg-rich smectite or mica in both Low-Ca and Skywalker. The phyllosilicates in the carbonaceous chondrites are commonly much better crystallized than those in the IDPs. Low-Ca and Skywalker are thus probably not broken fragments of carbonaceous chondrites, and they may well have originated from distinct sources.

Petrographical studies²⁶⁻³⁰ suggest that the CI and CM phyllosilicates were produced largely by aqueous alteration of olivines and pyroxenes. The present study provides evidence suggesting that the IDP phyllosilicates were also formed by alteration of Fe-Mg pyroxene. The differences in phyllosilicate mineralogy may then reflect the distinct environments in which the alteration occurred. If the alteration occurred within the parent bodies—and this is only an assumption at present—the contrast in mineralogy may reflect differences between comets and asteroids, the presumptive parent bodies of IDPs and carbonaceous chondrites^{31,32}.

The hydrated and non-hydrated IDPs have similar bulk compositions and isotope characteristics but different mineralogies. A question arises as to whether the distinct mineralogies reflect different environments. During or after accretion from the solar nebula, the IDPs may have experienced distinct physico-chemical conditions or different degrees of thermal metamorphism. To resolve this question, additional mineralogical and isotope data are required as well as a better understanding of the range of types of chondritic IDPs. Work is in progress towards achieving these goals.

We thank Dr R. M. Walker and his co-workers at Washington University for providing the Skywalker IDP and for helpful discussions. We also thank Drs D. E. Brownlee, R. Christoffersen and L. Grossman for useful discussions and Dr K. Keil for a helpful review. Electron microscopy was performed at the electron microscope facility in the Center for Solid State Science at Arizona State University. This work was supported by NASA grant NAG 9-59.

Received 4 October 1984; accepted 22 January 1985.

1. Sandford, S. A., Fraundorf, P., Patel, R. & Walker, R. M. *Meteoritics* **17**, 276-277 (1982).
2. Zinner, E. & McKeegan, K. D. *Lunar planet. Sci.* **15**, 961-962 (1984).
3. Zinner, E., McKeegan, K. D. & Walker, R. M. *Nature* **305**, 119-121 (1983).
4. Cliff, G. & Lorimer, G. W. *J. Microsc.* **103**, 203-207 (1975).
5. Tomeoka, K. & Buseck, P. R. *Earth planet. Sci. Lett.* **69**, 243-254 (1984).
6. Brownlee, D. E. in *Protostars and Planets* (ed. Gehrels, T.) 134-150 (University of Arizona Press, 1978).
7. Deer, W. A., Howie, R. A. & Zussman, J. in *Rock Forming Minerals* Vol. 2A, 399-414 (Wiley, New York, 1978).
8. Clarke, R. S. et al. *Smithson. Contr. Earth Sci.* **5**, 1-53 (1970).
9. Grossman, L. *Geochim. cosmochim. Acta* **39**, 433-454 (1975).
10. Macdougall, J. D. *Earth planet. Sci. Lett.* **42**, 1-6 (1979).
11. MacPherson, G. J., Bar-Matthews, M., Tanaka, T., Olsen, E. & Grossman, L. *Geochim. cosmochim. Acta* **47**, 823-839 (1983).
12. Bischoff, A. & Keil, K. *Nature* **303**, 588-592 (1983).
13. Bischoff, A. & Keil, K. *Geochim. cosmochim. Acta* **48**, 693-709 (1984).
14. Bradley, J. P., Brownlee, D. E. & Fraundorf, P. *Science* **226**, 1432-1434 (1984).
15. Fraundorf, P., Flynn, G. J., Shirck, J. & Walker, R. M. *Proc. 11th Lunar. planet. Sci. Conf.*, 1235-1249 (1980).
16. Iijima, S. & Buseck, P. R. *Am. Miner.* **60**, 758-770 (1975).
17. Buseck, P. R. & Iijima, S. *Am. Miner.* **60**, 771-784 (1975).
18. Christoffersen, R. & Buseck, P. R. *Lunar planet. Sci.* **15**, 152-153 (1984).
19. Bradley, J. P., Brownlee, D. E. & Veblen, D. R. *Nature* **301**, 473-477 (1983).
20. Veblen, D. R. & Buseck, P. R. *Am. Miner.* **66**, 1107-1134 (1981).
21. Eggleton, R. A. & Boland, J. N. *Clay Clay Miner.* **30**, 11-20 (1982).
22. Tomeoka, K. & Buseck, P. R. *Nature* **299**, 327-329 (1982).
23. Yada, K. & Ishii, K. *Am. Miner.* **62**, 958-965 (1977).
24. Fuchs, L. H., Olsen, E. & Jensen, K. J. *Smithson. Contr. Earth Sci.* **10**, 1-39 (1973).
25. Tomeoka, K. & Buseck, P. R. *Nature* **306**, 354-356 (1983).
26. DuFresne, E. R. & Anders, E. *Geochim. cosmochim. Acta* **26**, 1085-1114 (1962).
27. Nagy, B., Meinschein, W. G. & Hennessy, D. J. *Ann. N.Y. Acad. Sci.* **108**, 534-552 (1963).
28. Boström, K. & Fredriksson, K. *Smithson. Misc. Collect.* **151**, 1-39 (1966).
29. Bunch, T. E. & Chang, S. *Geochim. cosmochim. Acta* **44**, 1543-1577 (1980).
30. McSweeney, H. Y. *Geochim. cosmochim. Acta* **43**, 1761-1770 (1979).
31. Brownlee, D. E. *Rev. Geophys. Space Phys.* **17**, 1735-1743 (1979).
32. Gaffey, M. J. & McCord, T. B. in *Comets, Asteroids, Meteorites* (ed. Delsemme, A. H.) 199-218 (University of Toledo Press, 1977).
33. Yoder, H. S. & Tilley, C. E. *J. Petrol.* **3**, 342-532 (1962).

Magnetic reversals and mass extinctions

David M. Raup

Department of the Geophysical Sciences, University of Chicago,
Chicago, Illinois 60637, USA

Previous analyses of the time distribution of reversals of the Earth's magnetic field have yielded mixed results. Some authors have claimed significant periodicities of order 10^7 yr^{1,2} whereas others have reported failure to reject null hypotheses of random spacing at that scale³⁻⁶. Because of repeated suggestions that field reversal is linked to biological extinction⁷⁻¹², further analysis of the magnetic time series is appropriate. I present here the results of a study of the reversal record of the past 165 Myr. A stationary periodicity of 30 Myr emerges (superimposed on the non-stationarities already established by others⁵), which predicts pulses of increased reversal activity centred at 10, 40, 70, ... Myr BP.

The database is the compilation of magnetic reversals for the late Jurassic to present interval (0-165 Myr BP) by Harland *et al.*¹³. These data, constituting the magnetostratigraphical timescale for the best-known part of the reversal record, were culled heavily by Harland *et al.*¹³ to eliminate minor excursions and other short-term or local effects and were presented as the time boundaries of 148 magnetic intervals (chrons and subchrons), yielding $148 \times 2 = 296$ reversal events. Figure 1 shows the distribution of these events in geological time, which shows, among other things, the well-known quiet period of the late Cretaceous (83-118 Myr BP), with reversal activity decreasing before and increasing after the quiet period.

In developing the statistical methodology for this analysis, a magnetic reversal is assumed to be an isolated event; thus, it is not important whether the field shifted from normal to reversed or vice versa. The analytical procedure, which follows that of Stothers¹⁴ and Raup and Sepkoski¹⁵, has as a central feature a 'goodness-of-fit' metric applicable to any postulated stationary periodicity. This metric is calculated as follows. For a given trial period (such as 20 Myr), a predicted sequence of events is defined and placed on the timescale in a completely arbitrary phase position (such as -10, 10, 30, 50, ... Myr BP). Then, the fit is assessed as the standard deviation of departures of the observed events from the predicted event closest to each. Thus, in the hypothetical example used here, an event observed at 14 Myr BP would show a departure of +4 Myr from its closest predicted event (10 Myr BP), an observed event at 48 Myr BP would show a departure of -2 (from the 50-Myr BP prediction) and so on. The standard deviation of these departures is a measure-of-fit for a given phase position, which is then shifted by a small increment (to, for example, -9, 11, 31, 51, ... Myr BP) and the standard deviation of departures is recalculated.

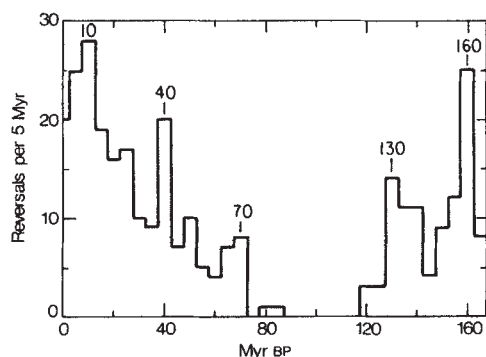


Fig. 1 Frequency of 296 magnetic reversals in 5-Myr intervals (data from ref. 13).

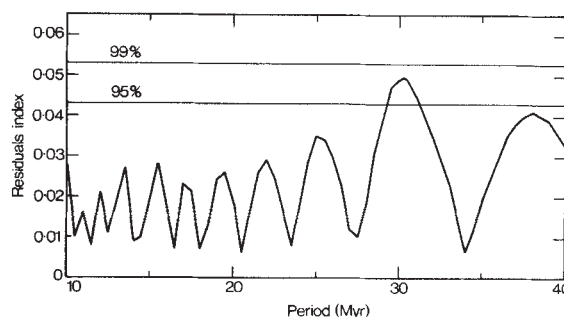


Fig. 2 Spectrum of residuals index ($n = 296$) constructed using the method of Stothers¹⁴. The residuals index is the measure of goodness of fit of the data to a given period.

This process is repeated for a series of phase increments (totaling at least a full period) and the lowest value of the standard deviation is taken as the goodness-of-fit. We thus have estimates of goodness-of-fit and best-fit position for the trial period. The method is particularly appropriate for time series exhibiting missing or missed data because waiting times between events are never examined explicitly. Also, the method contains no assumption that the frequency behaviour is sinusoidal, contrary to conventional Fourier techniques.

Stothers¹⁴ showed that the standard deviation can be normalized for period length as follows. Residuals index

$$(P\sqrt{(n^2 - 1)/12n^2 - \sigma})/P$$

where P is the period length, n is the number of observations and σ is the standard deviation of departures (above). With random data, the residuals index is constant over a range of P (but see below).

Given an observed time series, the residuals index may be calculated for a sequence of P values and a spectrum of goodness of fit produced, which is shown in Fig. 2 for the magnetic reversal time series; periods greater than 40 Myr are not included because the residuals index is unstable above $P = 40$ with this particular time series. There are a number of well-defined peaks, but this is not surprising; indeed, random input of the same general character often gives comparable spectra and so it is important to assess the probability that the spectrum is not just the product of chance. Using a bootstrap technique, the real-time series was randomized by scrambling the intervals between successive magnetic reversals; the first and last reversals were used as fixed endpoints and all intervals in between were rearranged by standard Monte Carlo techniques. Each randomized version of the data thus retained most of the fabric of the true series, but the placement of reversals was changed; each had a quiet period

Table 1 Goodness-of-fit and phase for magnetic reversal data

Period (Myr)	Goodness-of-fit: %				Phase in best-fit		
	of simulations with poorer				position: age of cycle		
	fit than the real data				no. 1		
	0-165	0-83	118-165	(Myr BP)	0-165	0-83	118-165
22	83.6	43.2	96.8		4.49	4.59	4.07
23	45.8	12.2	100.0		0.05	8.59	20.12
24	64.6	20.2	100.0		12.03	8.38	14.13
25	92.2	31.7	100.0		8.35	8.23	8.60
26	84.2	57.5	100.0		7.12	8.91	3.07
27	29.5	75.0	99.9		5.15	7.93	24.27
28	65.1	81.1	99.9		15.03	7.03	19.45
29	98.0	81.7	99.7		12.16	10.62	14.90
30	99.4	82.9	99.6		10.04	10.04	10.05
31	98.9	80.4	99.0		7.80	9.77	5.18
32	96.8	77.5	97.6		5.11	9.20	0.93
33	83.0	74.8	96.5		3.93	8.97	29.04

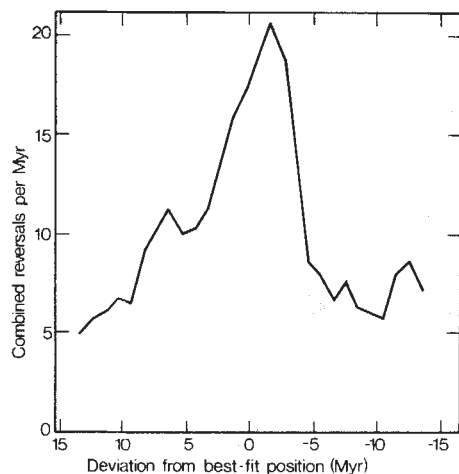


Fig. 3 Composite cycle of magnetic reversal activity plotted as a 3-point moving average. With a 30-Myr period in best-fit position (zero on the horizontal axis), the data from all cycles have been superimposed ($n = 296$).

of the same length, but its position was unpredictable. A spectrum, such as that in Fig. 2, was calculated for each of 200 simulations and the point of highest residuals index for each simulation was recorded. The results are summarized by the two horizontal lines in Fig. 2; 95 and 99%, respectively, of the simulated spectra were contained totally below the designated lines.

The peak corresponding to a period of 30 Myr stands out and is clearly above the 95% line, from which it can be concluded that it is unlikely that the peak is an accident or artefact of the method (Fig. 2). The null hypothesis in this case comes from the above randomization scheme. It is non-Poisson in that the distribution of intervals (waiting times) between events is maintained; only their order has been randomized. Tests with two alternative null hypotheses will be discussed below.

With the 30-Myr peak in Fig. 2 as a starting point, a more detailed investigation of periods in that region was undertaken. For each 1-Myr interval from 22 to 33 Myr, the fit of the real data (measured by the standard deviation of departures) was compared with that of 1,000 simulations of the type used earlier. The results are shown in columns 2 and 5 of Table 1. In column 2, goodness-of-fit is expressed as the percentage of simulations showing a poorer fit (higher standard deviation); the period at 30 Myr is the highest with 99.4%. Column 5 gives the best-fit position expressed as the geological age (in Myr BP) of the most recent pulse of reversal activity and this position for the 30-Myr period is 10.04 Myr BP.

To check these results, the analysis was repeated with non-overlapping segments of the time series, using the Cretaceous quiet period as the demarcation. These results are shown in the remaining columns of Table 1. For the younger segment (0–83 Myr BP), goodness-of-fit values are not as high as for the whole series but the one for 30 Myr is the highest. Also, the best-fit position is identical at 10.04 Myr BP. For the older segment (118–165 Myr BP) all periods show excellent fit, making it difficult to distinguish between them. This is not unreasonable because this segment is only 45 Myr long and contains only two of the events predicted by the complete time series. Note, however, that the best-fit position for the 30-Myr period estimated from this short segment is 10.05 Myr BP, essentially identical to the other estimates for this period. Thus, the analyses of segments can be regarded as confirming a 30-Myr period with a best-fit phase position of 10 Myr BP.

I conclude that the reversal time series probably shows a stationary periodicity predicting elevated reversal activity at 30-Myr intervals beginning at 10 Myr BP. Extrapolating from this conclusion, Fig. 1 shows that the record is dominated by the Cretaceous quiet period with reversal activity increasing in

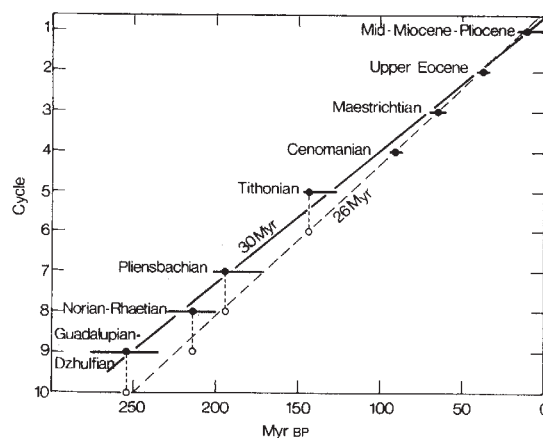


Fig. 4 The distribution by age and cycle number of the eight significant biological extinctions of the last 250 Myr (solid dots). Error bars indicate the maximum possible age spread, including that implied by the several available geological timescales²⁵. The solid diagonal corresponds to a 30-Myr period in the best fit position indicated by the magnetic reversal data. If the 26-Myr period is used, the oldest four extinctions must be advanced one cycle each (open circles) and the fit is improved substantially (dashed diagonal).

both directions. The 30-Myr periodicity is superimposed on this broader first-order change and can be seen as localized peaks at 10, 40, 70, 130 and 160 Myr BP; the predicted peak at 100 Myr BP is missing because of overall reduction in reversal activity (the quiet period).

I emphasize that a statistical confidence level cannot be assigned to the 30-Myr periodicity. Although the data clearly favour this periodicity over the null hypothesis of random ordering of the same data, an unknown number of stationary and non-stationary models might give as good or a better fit. The 30-Myr stationary model is, nevertheless, the simplest alternative to the random model and seems to be well supported by the data.

Confidence in the 30-Myr periodicity depends on the null hypothesis against which it is tested; it was therefore tested against two other hypotheses. The first is a simple Poisson model where randomization was achieved by 'dropping' 296 points on a 0–165-Myr timescale using uniformly distributed random numbers; the result was identical, within sampling error, to that reported in Table 1. The second variant reproduced the non-stationarities reported by McFadden and Merrill⁵. Once again randomization was achieved by a Poisson method, but in this case the linear decrease in reversal activity before the Cretaceous quiet period and the linear increase after that were used to bias the selection of simulated events. The probabilities used were drawn from linear fits to the actual data following the interpretation of McFadden and Merrill⁵. The result, identical to that reported in Table 1 (99.4% of simulations showing a poorer fit than the real data), confirms further the conclusion that the observed periodicity is superimposed on the larger-scale changes in reversal frequency.

Figure 3 shows the frequency distribution of reversal events in a single composite 30-Myr period. The relatively smooth and symmetrical distribution supports the hypothesis of a 30-Myr period with a best-fit position centred at 10, 40, 70, ... Myr BP. If other periods or phase positions are used to construct the composite, the distribution breaks down.

These results are in good agreement with other analyses^{1,2}. Using Walsh spectrum analysis applied to a much longer series of magnetic reversal data, Negi and Tiwari¹ recognized several periodicities and claimed statistical significance for those at 32 and 285 Myr. Mazaud *et al.*², working with the reversal data younger than the Cretaceous quiet period, found a 15-Myr periodicity, which is precisely in phase with the 30-Myr period reported here. Their strongest peaks occurred at 10 and 40 Myr BP and it is not clear (nor especially important) whether the 30-Myr period is a harmonic of the 15-Myr period or vice versa.

What is the relevance of these results to biological extinction? This question involves possible relationships between extinction, comet or asteroid impact and magnetic reversal as well as possible periodicities in all three. The link between extinction and magnetic reversal is supported by some studies⁷⁻¹² but challenged by others¹⁶⁻¹⁷; that between extinction and impact is more nearly established because of the iridium and/or other evidence for impact at four separate extinction times—late Eocene¹⁸⁻¹⁹, terminal Cretaceous²⁰⁻²¹, terminal Permian²² and late Devonian²³. Further, the suggestion that extinctions and impact events are both periodic and in phase^{12,24-26} strengthens the case for causal linkage. A proposed link between large-body impact and magnetic reversal is more tenuous, but is supported by some evidence, specifically the temporal coincidence of magnetic reversals with three of the four well-dated tectite-strewn fields²⁷. It is thus not impossible that at least some reversals are caused by comet or asteroid impact and it is in this context that the relationship between periodic intensification of reversal activity and periodic extinction becomes important.

For the most recent several cycles, the fit between extinctions and reversal intensity is reasonable in view of the extinctions at 11.3, 38 and 65 Myr BP²⁵ and the magnetic reversal peaks at 10, 40 and 70 Myr BP. But because the most probable periodicity for extinctions is 26 Myr^{15,25}, the two systems move out of phase going backwards in time. This is illustrated in Fig. 4, which shows an attempt to fit the 30-Myr periodicity to the eight significant extinctions of the past 250 Myr and shows also the 26-Myr fit to the same extinction points. Except for the first three events, the discrepancy between the two periods is clearly non-trivial.

The discrepancy can mean either there are independent periodicities operating with no causal connection or that the statistical uncertainty of the analyses is large enough to accommodate a single period somewhere in the 26–30-Myr range. The situation is complicated further by the fact that Rampino and Stothers¹² re-analysed the extinction data and found a best-fit period of 30 ± 1 Myr, with phase position at 10 ± 7 -Myr BP. They used a regression method that assumes that all cycles are present in the data, although they noted that removing this restriction restores the 26-Myr period of Raup and Sepkoski^{15,25}.

The discrepancies in analytical results mean that no unequivocal conclusion can be drawn; however, there is a possibility that biological extinction, magnetic reversal and large-body impact are linked.

I thank M. R. Rampino, J. J. Sepkoski Jr, S. Stigler and R. B. Stothers for discussions and also G. E. Boyajian for literature search and for help with the analysis of the magnetic data. The work was supported by NASA grant NAG 2-237.

Received 21 September 1984; accepted 22 January 1985.

1. Negi, J. G. & Tiwari, R. K. *Geophys. Res. Lett.* **10**, 713–716 (1983).
2. Mazaud, A., Laj, C., de Sèze, L. & Verosub, K. L. *Nature* **304**, 328–330 (1984).
3. Phillips, J. D. & Cox, A. *Geophys. J. R. astr. Soc.* **45**, 19–33 (1976).
4. Cox, A. *Phys. Earth Planet. Interiors* **24**, 178–190 (1981).
5. McFadden, P. L. & Merrill, R. T. *J. geophys. Res.* **89**, 3354–3362 (1984).
6. McFadden, P. L. *Nature* **311**, 396 (1984).
7. Uffen, R. J. *Nature* **196**, 143–144 (1963).
8. Simpson, J. F. *Bull. geol. Soc. Am.* **77**, 197–204 (1966).
9. Hatfield, C. B. & Camp, C. J. *Bull. geol. Soc. Am.* **81**, 911–914 (1970).
10. Hays, J. D. *Bull. geol. Soc. Am.* **82**, 2433–2447 (1971).
11. Crain, I. K. *Bull. geol. Soc. Am.* **82**, 2603–2606 (1971).
12. Rampino, M. R. & Stothers, R. B. *Nature* **308**, 709–712 (1984).
13. Harland, W. B. *et al.* *A Geologic Time Scale* (Cambridge University Press, 1982).
14. Stothers, R. B. *Astr. Astrophys.* **77**, 121–127 (1979).
15. Raup, D. M. & Sepkoski, J. J. *Proc. natn. Acad. Sci. U.S.A.* **81**, 801–805 (1984).
16. Thomson, K. S. in *Patterns of Evolution* (ed. Hallam, A.) 377–404 (Elsevier, Amsterdam, 1977).
17. Plotnick, R. E. *Geology* **8**, 578–581 (1980).
18. Ganapathy, R. *Science* **216**, 885–886 (1982).
19. Alvarez, W., Asaro, F., Michel, H. V. & Alvarez, L. W. *Science* **216**, 886–888 (1982).
20. Alvarez, L. W., Alvarez, W., Asaro, F. & Michel, H. V. *Science* **208**, 1095–1108 (1980).
21. Alvarez, W. *et al.* *Science* **223**, 1135–1141 (1984).
22. Sun, Y. *et al.* in *Developments in Geoscience*, 235–245 (Academia Sinica, Science Press, Beijing, 1984).
23. Playford, P. E., McLaren, D. J., Orth, C. J., Gilmore, J. S. & Goodfellow, W. D. *Science* **226**, 437–439 (1984).
24. Alvarez, W. & Muller, R. A. *Nature* **308**, 718–720 (1984).
25. Sepkoski, J. J. & Raup, D. M. in *Dynamics of Extinction* (ed. Elliott, D.) (Wiley, New York, in the press).
26. Rampino, M. R. & Stothers, R. B. *Science* **226**, 1427–1431 (1984).
27. Glass, B. P., Swincki, M. B. & Zwart, P. A. *Proc. 10th Lunar Planet. Sci. Conf.* 2535–2545 (1979).

Lead isotope differences between whole-rock and phenocrysts in recent lavas from southern Italy

M. Cortini* & P. W. C. van Calsteren†

* Istituto di Geologia & Geofisica, University of Naples, 80138 Naples, Italy

† Department of Earth Sciences, Open University, Milton Keynes MK7 6AA, UK

In southern Italy, there are several active volcanoes which occur in diverse geotectonic settings. Isotope determinations on coexisting minerals and whole-rocks from zero-age lavas from Vesuvius, Etna and Stromboli reveal an isotope equilibrium for Sr isotopes¹ but not for Th (ref. 2). Analyses for Pb in the same samples also reveal differences in isotope composition, and in all cases the phenocrysts are less radiogenic than the whole-rocks. We argue here that the Pb and Th isotope composition of the magmas changed during and after fractional crystallization, possibly by crustal assimilation or by addition of mantle-derived fluids and that whole-rock Pb isotope data are not representative of the magma sources.

Stromboli forms part of the Aeolian island arc, probably along a destructive margin between the European and African plates. It is characterized by virtually uninterrupted eruption of leucite tephrites and basaltic andesites belonging to the high-K-calc-alkaline series. Model calculations based on trace elements indicate an origin by partial melting of a parent material inhomogeneously enriched by a Th/light rare-earth element/P-rich metasomatic fluid, probably derived from the subducting plate³.

Etna is a large alkali basalt volcano, with minor early tholeiitic lavas, producing mainly hawaiites and mugearites. Its geochemistry is largely consistent with its within-plate setting but a minor modification of its source by subduction-derived phases is likely³.

Vesuvius is best known for its explosive-effusive eruptions of high-K trachytes and leucite tephrites; they probably derive from a comparatively recently enriched mantle source, by analogy with nearby Roccamonfina⁴. Fractional crystallization and cumulus formation took place in various magma chambers at different levels in the crust; the incorporation of limestone fragments, high in the crust, had little effect on the volcanic rocks.

Table 1 shows the results of our analyses of the Pb isotope composition of whole-rock and mineral separates of lavas and ejected cumulates of well-studied samples. Separated minerals are either phenocrysts, considered to have crystallized from the coexisting magma, or biotite separated from nodules which have been interpreted as cumulates⁵.

Pb was separated using a 0.1-ml AG 1×8 ion-exchange column, eluted with 1M HBr and stripped with 2 ml H₂O. Pb was loaded on a Re filament with phosphoric acid and silica gel. Isotope ratios were measured using our software on a VG54E mass spectrometer. The NBS 981 standard was run in the same conditions and from the collated results a bias factor of 1.00047 AMU^{-1} was calculated. Measured results were corrected using this factor to minimize the effects of instrumental mass fractionation. The total procedure blank is <2 ng; sample weights were adjusted to contain an estimated 1 µg Pb.

Our data for the Vesuvius 1944 lava are in agreement with Vollmer and Hawkesworth⁶, while those from the Etna lavas compare well with the data of Carter and Civetta⁷. Reproducibility may be estimated from the two duplicate whole-rock analyses.

There are no significant differences in Sr isotope ratios (Table 1) between phenocrysts and their whole-rocks, indicating that phenocrysts are indeed co-magmatic. Differences in Sr isotopes between Vesuvius samples from different magma batches can be explained by mixing two endmembers with very similar major-element, but different isotope, compositions¹.

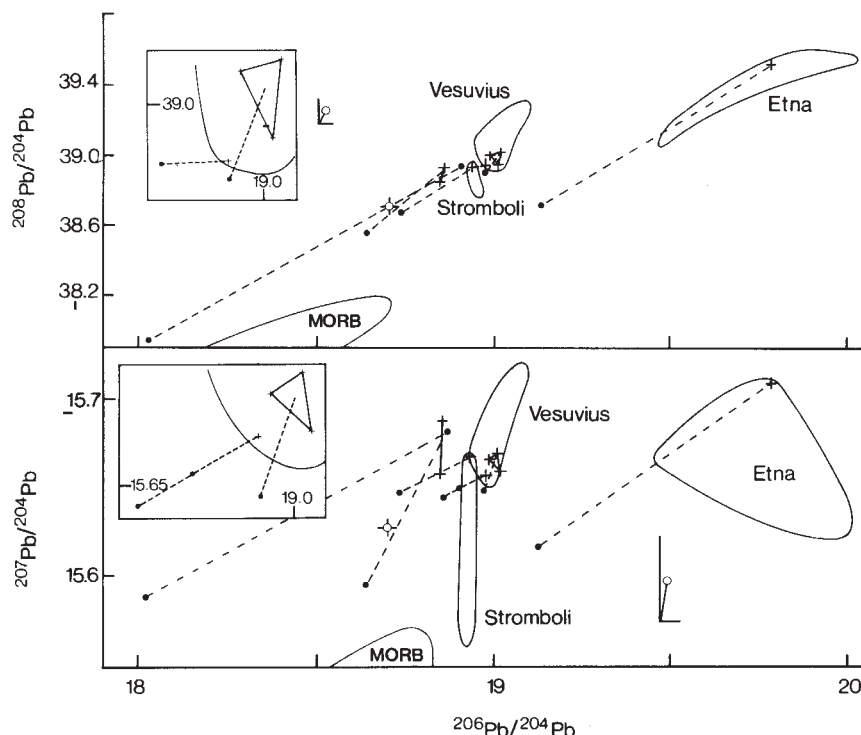


Fig. 1 Pb isotope diagram. Solid lines connect whole-rock duplicates; dashed lines tie whole-rock and mineral. Inset, Vesuvius lava data on a larger scale. +, Average modern Pb^{16} . $\perp$, The error on replicate analyses of NBS 981 and the applied correction. Top of MORB field from ref. 17.

Pb isotope compositions are plotted in Fig. 1 together with fields constructed using the present data and some from other workers⁶⁻⁹. All phenocryst and cumulate nodule data plot away from the whole-rock fields towards less radiogenic compositions. The largest displacement of plagioclase with respect to the whole-rock from which it was separated is from Etna, and is well outside the experimental error. Deviations for the Stromboli and Vesuvius-Castello di Cisterna pairs are smaller but still significant. Data for the Vesuvius 1944 lava are strictly within error, but display a similar relation.

In the case of the cumulate nodules, there are also large differences between biotite and whole-rock analyses. These data suggest that the Pb isotope composition of the respective magmas changed during and after fractional crystallization, although the Sr isotope composition was not affected. (For Sr, a difference in isotope composition caused by mass-dependent fractionation would be eliminated by the standard correction procedure as

its effect is indistinguishable from the instrumental fractionation.) Mass-dependent Pb isotope fractionation effects will be parallel to the NBS 981 correction vector. Clearly, none of the mineral, whole-rock tie-lines are parallel to this vector and the most probable explanation is mixing of two components with different Pb isotope composition. The actual nature of the components is difficult to assess but it can be inferred that the magma source had Pb isotope ratios even less radiogenic than indicated by the phenocrysts or cumulate minerals but probably still more radiogenic than mid-ocean ridge basalts (MORB). Slopes of tie-lines on Fig. 1 between minerals and whole-rocks are ~ 0.12 , suggesting an age of $\sim 2,000$ Myr for the radiogenic component. Our data do not indicate whether the mixing process involved bulk assimilation, exchange of Pb through interaction with fluids or addition of Pb by metasomatic fluids in the mantle. However, for Vesuvius, fractional crystallization has probably taken place in the mantle at pressures greater than the stability limit of

Table 1 Pb isotope composition compared with Sr and Th data for various Italian lavas

		$^{206}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{208}Pb/^{204}Pb$	$^{207}Pb/^{206}Pb$	$^{87}Sr/^{86}Sr$	$^{230}Th/^{232}Th^*$
Stromboli	WR	18.931 ± 5	15.668 ± 5	38.945 ± 11	0.82768 ± 4	$0.70624 \pm 4^\dagger$	0.92 ± 1
1975 lava Stromboli ¹³	Plag	18.734 ± 2	15.641 ± 1	38.685 ± 4	0.83490 ± 2	$0.70621 \pm 4^\ddagger$	0.92 ± 6
Etna	WR	19.777 ± 3	15.712 ± 3	39.546 ± 9	0.79445 ± 2	0.70350 ± 6	0.98 ± 2
1971 lava Etna ¹⁵	Plag	19.129 ± 20	15.617 ± 14	38.726 ± 40	0.81638 ± 8	0.70343 ± 4	1.34 ± 10
Vesuvius/Somma	WR	19.016 ± 1	15.660 ± 1	39.041 ± 4	0.82353 ± 1	$0.70718 \pm 3^\S$	0.86 ± 2
	WR	18.978 ± 2	15.667 ± 2	39.030 ± 6	0.82553 ± 2		
1944 lava Vesuvius	WR	19.008 ± 13	15.671 ± 11	38.969 ± 36	0.82431 ± 7		
	Leuc	18.969 ± 7	15.648 ± 6	38.930 ± 18	0.82489 ± 3	$0.70718 \pm 3^\S$	1.10 ± 9
Castello di Cisterna lava	WR	18.967 ± 1	15.659 ± 1	38.947 ± 4	0.82557 ± 5	$0.70761 \pm 4^\S$	
	Leuc	18.857 ± 11	15.646 ± 10		0.82971 ± 1	$0.70757 \pm 6^\S$	
	Leuc	18.906 ± 3	15.652 ± 2	38.944 ± 6	0.82785 ± 2		
Cumulate	WR	18.841 ± 1	15.650 ± 1	38.870 ± 2	0.83065 ± 1	$0.70741 \pm 5^\S$	1.14 ± 4
N1	WR	18.854 ± 5	15.689 ± 5	38.950 ± 12	0.83215 ± 4		
	Biot	18.636 ± 1	15.595 ± 10	38.571 ± 22	0.83681 ± 4	$0.70761 \pm 5^\S$	1.25 ± 6
Cumulate	WR					$0.70728 \pm 5^\S$	1.02 ± 6
N83	Biot	18.028 ± 6	15.588 ± 5	37.947 ± 13	0.86554 ± 3	$0.70766 \pm 3^\S$	1.33 ± 10
	Biot	18.861 ± 3	15.682 ± 3	38.952 ± 6	0.83148 ± 3		
NBS 981		16.923 ± 6	15.470 ± 7	36.650 ± 16	0.91417 ± 10		
N = 14							

Errors are 1σ for within-run precision; for NBS 981 it is the standard deviation. Samples are corrected for fractionation using a factor of 1.00047 AMU^{-1} derived from repeated analyses of NBS 981. WR, whole-rock; Plag, plagioclase; Leuc, leucite; Biot, biotite.

* Activity ratios from refs 2, 10, 11. Sr isotope data from † ref. 10, ‡ ref. 14, § ref. 1.

plagioclase⁵. Thus, a mantle component is probably involved. Furthermore the ²³⁰Th/²³²Th activity ratios^{2,10,11} summarized in Table 1 also show differences in isotope composition between whole-rock and minerals in most cases. Capaldi *et al.*² have argued that these differences do not have any significance for the age but indicate open system behaviour, and they propose contamination of the magmas with mantle fluids. This must have been a recent event because the high decay rate of the ²³⁸U daughter products¹² involved will establish a new equilibrium in 0.2 Myr.

The observed differences in mineral/whole-rock Pb isotopes also imply that whole-rock data alone are not representative of the magma sources, at least in the cases of Vesuvius, Stromboli and Etna. The observed variations suggest that the magma sources are less radiogenic than the whole-rock and probably even the mineral data.

We thank Nick Rogers and Chris Hawkesworth, for useful comments, John Taylor for drawing the diagram and Carol Whale for typing the manuscript. Work at the Open University by M.C. was funded by a NATO fellowship grant.

Received 18 October 1984; accepted 22 January 1985.

1. Cortini, M. & Hermes, O. D. *Contr. Miner. Petrol.* **77**, 47–55 (1981).
2. Capaldi, G., Cortini, M. & Pece, R. *J. volcan. geotherm. Res.* **14**, 247–260 (1982).
3. Beccaluva, L., Rossi, P. L. & Serri, G. *Earth Evol. Sci.* **3**, 222–238 (1982).
4. Hawkesworth, C. J. & Vollmer, R. *Contr. Miner. Petrol.* **69**, 151–165 (1979).
5. Hermes, O. D. & Cornell, W. C. *NSF Final Tech. Rep.* (Univ. Rhode Island, 1978).
6. Vollmer, R. & Hawkesworth, C. J. *Earth planet. Sci. Lett.* **47**, 91–101 (1980).
7. Carter, S. R. & Civetta, L. *Earth planet. Sci. Lett.* **36**, 168–180 (1977).
8. Cortini, M. *Bull. volca.* **44–4**, 711–722 (1981).
9. Vollmer, R. *Geochim. cosmochim. Acta* **40**, 283–295 (1976).
10. Capaldi, G., Cortini, M. & Pece, R. *Isotope Geosci.* **1**, 39–55 (1983).
11. Capaldi, G. & Pece, R. *J. volcan. geotherm. Res.* **11**, 367–372 (1981).
12. Capaldi, G. *et al.* *J. geophys. Res.* **81**, 350–358 (1976).
13. Barberi, F., Gasparini, P., Innocenti, F. & Villari, L. *J. geophys. Res.* **78**, 5221–5232 (1973).
14. Vollmer, R., Johnston, K., Ghiara, M. R., Lirer, L. & Munno, R. *J. volcan. geotherm. Res.* **11**, 317–327 (1981).
15. Di Girolamo, P. *Bull. volca.* **41–3**, 1–22 (1978).
16. Stacey, J. S. & Kramers, J. D. *Earth planet. Sci. Lett.* **26**, 207–221.
17. Sun, S. S. *Phil. Trans. R. Soc. A297*, 409–445 (1980).

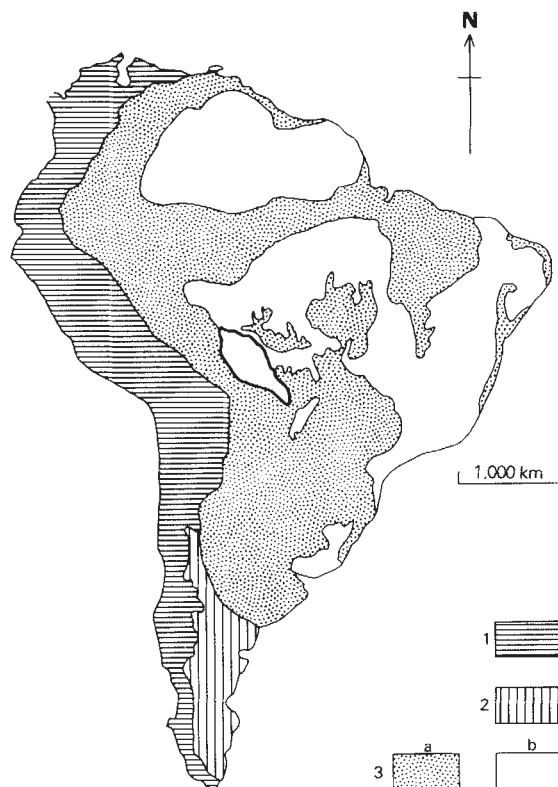


Fig. 1 The major geotectonic regions of South America¹¹ showing: (1) Andean Chain; (2) Patagonia Platform; (3) South America Platform—a, Phanerozoic cover; b, Brazilian Shield. The eastern Bolivia mapping area is in bold outline.

Andean-trending mobile belts in the Brazilian Shield

M. Litherland, B. A. Klinck, E. A. O'Connor & P. E. J. Pitfield

British Geological Survey, Keyworth, Nottingham NG12 5GG, UK

Much of the Phanerozoic Andean mountain chain is floored by Precambrian basement^{1–3}, in places as old as 2,000 Myr^{4,5}, but relations between these rocks and those of the Brazilian Shield have long been obscure, mainly because of the intervening Cenozoic sedimentary cover. We describe here recent work over the Bolivian sector of the Brazilian Shield (Fig. 1) which shows a pattern of Proterozoic mobile belts approximately parallel with the Andean chain to the west. When the trends of these Bolivian belts are projected towards the Andes under the Cenozoic cover, their ages broadly accord with those of the Andean basement. Our interpretation is that the two domains are linked in the form of a chain of five Andean-trending mobile belts with a combined width of ~1,000 km, becoming progressively younger to the west with ages ranging from 1,300 Myr to the present.

An Anglo-Bolivian mineral exploration project has completed recently the reconnaissance mapping of the Bolivian Precambrian shield (refs 6–8). The outcrop pattern is determined largely by the San Ignacio Orogeny⁷, a medium- to high-grade tectono-metamorphic event, which established a complex of granulites, gneisses and metasedimentary schist belts as well as extensive syn- to post-tectonic granites dated by Rb/Sr at 1,350–1,300 Myr BP (ref. 6 and M.L. *et al.*, in preparation) (Fig. 2). The protolith rocks of the granulites are considered to be a thick sedimentary sequence and a calc-alkaline plutonic suite, which Rb/Sr studies⁶ indicate to be at least 2,000 Myr old, the age of the Trans-Amazonian Orogeny⁹. The semipelitic gneisses, in part

higher-grade equivalents of the schist belt, are composed of a metamorphosed sequence of pelites and psammities, with minor volcanics, mafic igneous sills, ironstones and graphitic and calcareous sediments⁷. Palaeocurrent directions preserved in low-grade belts indicate a northerly provenance, from the direction of the present Amazonian Craton⁶ (Fig. 3). The schist belt sequence is thought to belong to a San Ignacio Cycle of deposition following the Trans-Amazonian Orogeny⁶.

The trend of the San Ignacio Belt is defined by the upright third phase of folding (Fig. 3), which was accompanied by penetrative deformation and the generation of the syntectonic granites⁶. This event folded the granulite-gneiss-schist metamorphic sequence established during the earlier sub-horizontal tectonic phase of the Orogeny⁶.

It is now proposed that the limit of the San Ignacio Orogeny in Brazil is the Ciriquiqui arc of mafic/ultramafic intrusives¹⁰ (Fig. 3) which follows the San Ignacio trend. North-east of this line there are relatively undeformed supracrustal sequences, the Beneficente Group and the Roosevelt Volcanic Formation (Fig. 2), dated at 1,600 Myr BP¹⁰, which rest unconformably on older Xingu Complex basement. These supracrustal units belong¹¹ to a 1,700–1,400-Myr BP phase of faulting, volcanism, plutonism and sedimentation. They are cut by the anorogenic 1,300-Myr BP Providencia Granites¹⁰ (Fig. 2), accompanied by a widespread K/Ar resetting of older dates, the Madeira event¹¹, a cratonic reactivation which we relate to the effects of the adjacent San Ignacio Orogeny. Other Brazilian geologists^{12,13} classify these same 1,700–1,400-Myr BP rocks as part of the Rio Negro-Juruena Belt, interpreted¹⁴ as a magmatic arc accreted to an older Amazonian nucleus to the north-east.

When the San Ignacio Belt is projected along its west north-west to north-west course under the Cenozoic cover (Fig. 4), it can be linked to similar gneisses and granulites found as massifs in the Columbian Andes which give Rb/Sr and U/Pb dates of 1,300–1,200 Myr BP¹⁵. Rocks of the Brazilian Shield to the east of this area have undergone the Parguazan reworking of

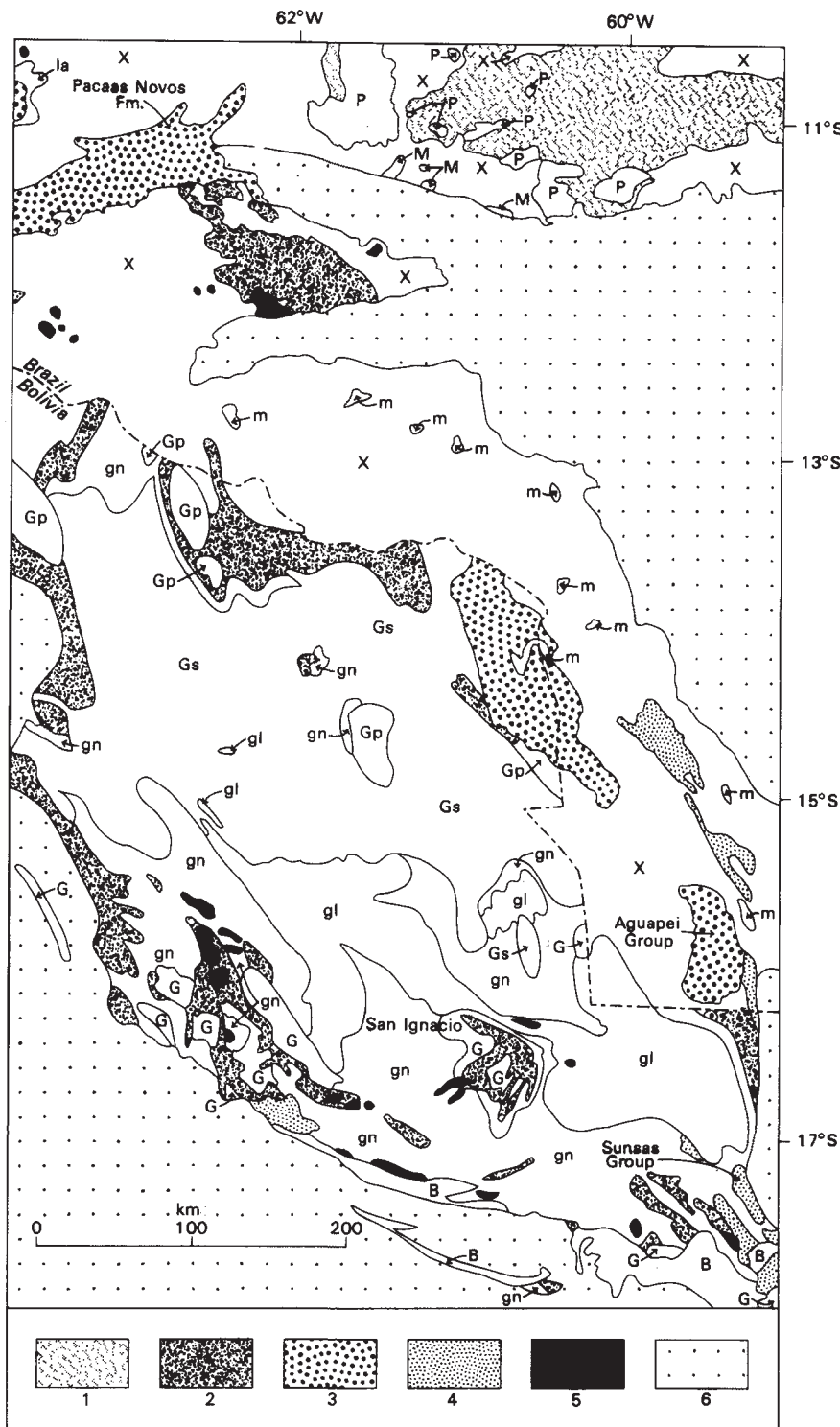


Fig. 2 Precambrian geology of eastern Bolivia^{6,8} and adjacent Brazil²⁶; tectonic contacts and Mesozoic igneous units omitted. The protolithic rocks of the following are all $\geq 1,400$ Myr BP: gl = granulites; gn = gneisses; X = Xingu Cx; (1) platform Beneficiente Group and Roosevelt Volcanic Formation; (2) San Ignacio schist belts. Of $\sim 1,300$ Myr BP: M = mafic/ultramafic intrusives; P = anorogenic Providencia Granites; G = orogenic granites (Gs, syntectonic; Gp, post-tectonic). Of 1,300–1,000 Myr BP: (3) platform Sunsas Group; (4) mobilized Sunsas Group; la = basaltic lavas. Of $\sim 1,000$ Myr BP: m = mafic/ultramafic intrusives; (5) Rondonia/Sunsas granites. B = Brasiliano Cycle sediments (~ 600 Myr BP); (6) Phanerozoic cover.

1,500 Myr BP¹⁶ and also exhibit the $\sim 1,300$ -Myr BP K/Ar resetting known locally as the Nickerie event¹⁵.

Traced southwards through the Bolivian shield area the fold trends of the San Ignacio Belt swing from east south-east through south-east to south (Fig. 3). This is a primary swing and unrelated to secondary deformation of San Ignacio Orogeny age⁶; it can even be identified across the later Sunsas shear zones⁷ (Fig. 3). Further south, the San Ignacio Belt is projected west of the 1,700-Myr BP Rio Apa Complex¹⁷ (Fig. 4), but its continuation through Paraguay is conjectural.

The 1,300-Myr BP San Ignacio Orogeny was followed by a period of clastic dominantly-shallow-water deposition, forming the Sunsas and Vibosi Groups in Bolivia^{6,7} and their Brazilian correlatives, the Aguapei Group and Pacaas Novos Formation¹⁰,

which are locally intercalated with basaltic lavas (Fig. 2). Thicknesses are in the range of 800–5,000 m and there is always a marked basal unconformity. Palaeocurrent directions indicate a northerly source and there are similarities in volcanoclastic suites with those of the older San Ignacio schist belt psammities. Outcrops of this regional unit occur as prominent ridges, representing metamorphosed synformal infolds, or as mesas of flat-lying relatively-undisturbed strata. These can be used as lithostratigraphical indicators in elucidating the tectono-metamorphic history of the Sunsas Orogeny, dated by Rb/Sr and K/Ar at 1,000 Myr BP⁶. Two mobile belts can be recognized separated by a cratonic zone of undisturbed basement capped by Sunsas Group mesas (Figs 2 and 3). The west north-west trending Sunsas Belt is bounded and segmented by curvilinear

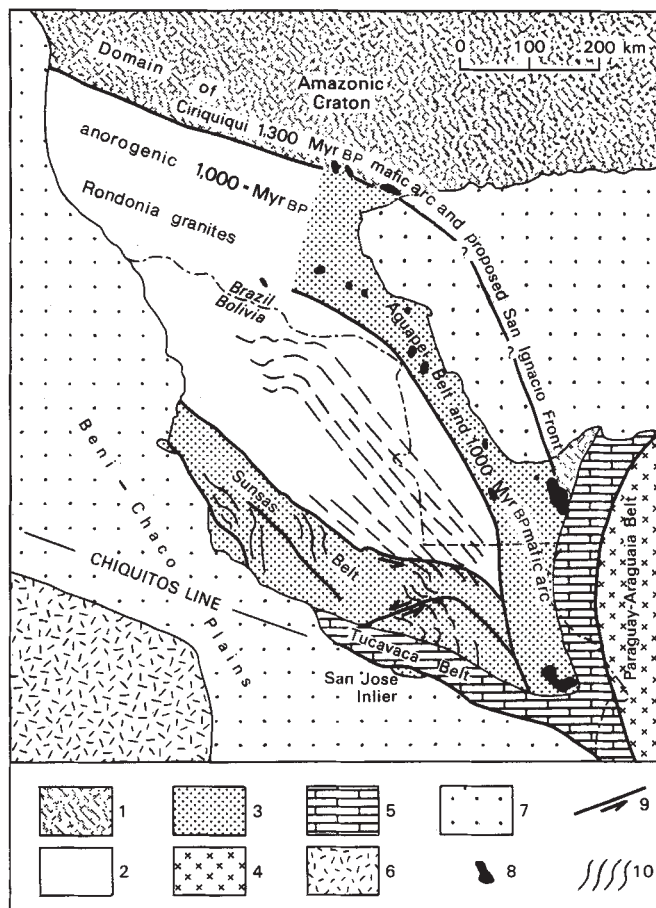


Fig. 3 Geotectonic elements of eastern Bolivia and environs. (1) Cratonic nucleus ($\geq 1,400$ Myr BP); (2) San Ignacio Mobile Belt (1,300 Myr BP); (3) 1,000-Myr BP mobile belts; (4) Brasiliano mobile belt (600–500 Myr BP); (5) Brasiliano platform cover; (6) Andean chain; (7) main areas of Phanerozoic platform cover; (8) mafic/ultramafic intrusions; (9) proposed shear zone or tectonic front, arrow indicates sense of horizontal movement; (10) San Ignacio fold trends.

sinistral shear zones which enclose sectors marked by coeval upright folds, metamorphism to garnet-grade and a penetrative deformation which cuts earlier recumbent 'thin-skin' folds in the Sunsas Group. Syn- and post-tectonic granites were developed accompanied by tin- and tantalum-bearing pegmatites^{7,8}. The Aguapei Belt (Fig. 3), in contrast, is a domain of faulting, folding and mafic-ultramafic intrusives. It seems to deflect the Sunsas shears in a dextral sense (Fig. 3) and follow the trend of the older San Ignacio Belt.

Tracing the Aguapei Belt to the north-west, the folding dies out and the mafic/ultramafic intrusives give way to the domain of the 1,000-Myr BP anorogenic Rondonia granites¹⁸ (Fig. 3). When the Sunsas Belt is projected west north-west to the Andes (Fig. 4), however, it lines up with probable 1,000-Myr BP basement rocks in Ecuador⁷. Sunsas Orogeny-age rocks also floor sectors of the Andes in Bolivia¹⁹ and Peru¹ and may represent older nuclei of an extensive Sunsas Belt within the proposed Brasiliano Belt (Fig. 4).

The Upper Proterozoic–Eocambrian Brasiliano (Pan-African) Cycle of the eastern Bolivian shield is restricted to the western fringe of the eugeosynclinal Paraguay–Araguaia Belt¹¹ and the west north-west-trending Tucavaca Belt of miogeosynclinal rocks⁷ (Fig. 3), bordered to the south by a tectonic front giving reset K/Ar dates of 520 Myr BP⁶ in the San José inlier (Fig. 3). The Brasiliano sequences extend under the Phanerozoic cover further south to link up²⁰ with the Andean-trending 600–500-Myr BP Brasiliano belt recognized in the Sierra Pampeanas of

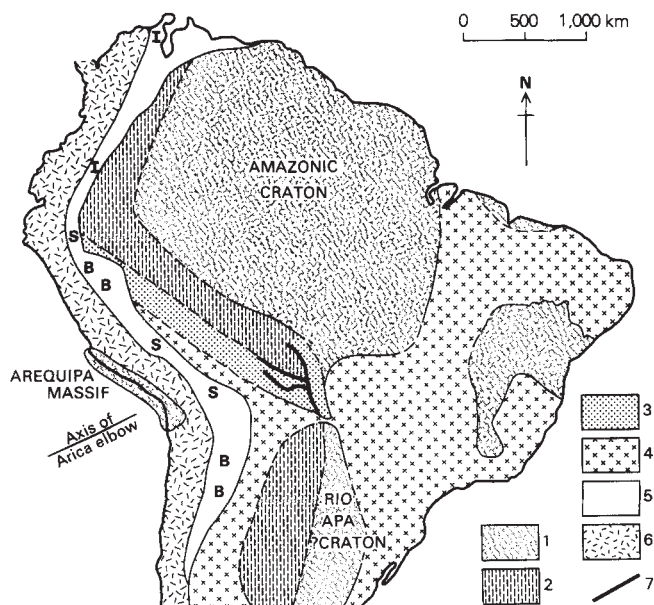


Fig. 4 Present interpretation of main South American geotectonic units; dashed lines indicate projection below South American Platform cover¹¹. (1) Cratonic nuclei ($\geq 1,400$ Myr BP); (2) San Ignacio Mobile Belt (1,300 Myr BP); (3) Sunsas and Aguapei Mobile Belts (1,000 Myr BP); (4) Brasiliano Mobile Belts (600–500 Myr BP); (5) Eastern Cordillera (Palaeozoic); (6) Western Cordillera (Mesozoic–Cenozoic); (7) 1,000-Myr BP shear zone. I, S and B refer respectively to San Ignacio, Sunsas and Brasiliano orogenic ages of sub-Andean basement rocks. Partially reworked tracts of older rocks within Brasiliano belt terrain¹¹ are omitted.

Argentina²¹ and other sectors of the sub-Andean basement^{1,3} (Fig. 4). The Tucavaca front was reactivated during the Phanerozoic as the Chiquitos Line²² (Fig. 3) controlling the distribution of Palaeozoic, Cretaceous and Cenozoic sedimentary basins⁶.

The Phanerozoic Andean chain can be divided into the Palaeozoic fold belt of the Eastern Cordillera and the Mesozoic–Cenozoic belt of Andean orogeny of the Western Cordillera^{1–3}. Figures 3 and 4 illustrate the parallelism of the Bolivian San Ignacio and Aguapei Belts with the Arica 'elbow': the major swing from north to west north-west in the trends of both the eastern and western Cordilleras parallel to the continental margin. There is also a parallelism between the west north-west-trending segment of the elbow and the Sunsas and Tucavaca Belts. The projected continuation of the belts away from the Bolivian outcrop (Fig. 4) accords broadly with the known Precambrian ages of the Andean basement. Thus, an enlarged Andean chain, ~1,000 km wide, can be proposed, consisting of five mobile belts which become progressively younger to the west towards the present continental margin. There is the possibility of an even older sixth belt within the Amazonian Craton (Fig. 4), in the form of the Paraguayan reworking of 1,500 Myr BP¹⁶ representing the north-west sector of the Rio Negro–Juruena Belt¹³. Whether such apparent continental growth layers relate to accretion of new crust or reworking of old must depend on the status of the 2,000-Myr BP Arequipa Massif⁴ (Fig. 4). Palaeomagnetic studies⁵ suggest it is not an allochthonous post-Jurassic microplate but an integral part of the American plate, continuous beneath the Andes with the Brazilian Shield and possibly extending further west before the onset of Mesozoic closure/subduction¹. If its position with respect to the Amazonian Craton has not changed since 2,000 Myr BP, then all five belts are probably ensialic, with continental margin subduction beginning in the Phanerozoic. However, if the Massif arrived after that time, then the San Ignacio Belt could have developed along a pre-Pacific oceanic suture. In either case, it appears that the trend of the

1,300-Myr BP San Ignacio Belt has controlled those of subsequent orogenies as well as the present shape of the continental margin, suggesting a longstanding tectonic regime established within the continent and perhaps related to the formation of the Pacific Ocean.

The identification of Proterozoic elements in an enlarged Andean chain would augment, at least locally, the number of peripheral mobile belts which young towards the southern continental margin of Gondwanaland²³, with implications regarding the mechanisms of formation, such as accretion or reworking²⁴, of the Brasiliano (Pan-African) belts. Traced to North America, the 1,300-Myr BP San Ignacio Belt may deviate from the circum-

Pacific chain to link up¹⁵ with the Grenville Belt of the North Atlantic chain²⁵.

The field work in eastern Bolivia was carried out with colleagues at the British Geological Survey (BGS) and counterparts at GEOBOL under a technical cooperation agreement funded by the Overseas Development Administration, Foreign and Commonwealth Office. D. P. F. Darbyshire (BGS) was responsible for the age determination programme. This letter is published with the permission of the Directors of BGS and the Servicio Geológico de Bolivia (GEOBOL), La Paz. We thank Dr E. J. Cobbing, Professor U. G. Cordani and Dr B. G. N. Page for helpful criticism.

Received 7 November 1984; accepted 28 January 1985.

1. Dalmayrac, B., Laubacher, G. & Marocco, R. *Caractères généraux de l'Évolution géologique des Andes péruviennes* (Office de la Recherche Scientifique et Technique Outre-Mer, Paris, 1980).
2. Cobbing, E. J. in *Ocean Basins and Margins* (Plenum, New York, in the press).
3. Zeil, W. *The Andes, A Geological Review* (Gebrüder Borntraeger, Berlin, 1979).
4. Cobbing, E., Ozard, J. M. & Snelling, N. J. *Bull. geol. Soc. Am.* **88**, 9-17 (1977).
5. Shackleton, R. M., Ries, A. C., Coward, M. P. & Cobbold, P. R. *J. geol. Soc.* **136**, 195-214 (1979).
6. *Unpublished Proyecto Precambrio final reports* Open file, GEOBOL (Bolivia) and BGS (Keyworth, Nottingham UK).
7. Litherland, M. & Bloomfield, K. *Precambrian Res.* **15**, 157-179 (1981).
8. Berrangé, J. P. *Episodes* **4**, 3-8 (1982).
9. Cordani, U. G., Amaral, G. & Kawashita, K. *Geol. Rdsch.* **63**, 9-17 (1973).
10. Leal, J. W. L. *et al. Proyecto Radambrasil 16* (Departamento Nacional da Produção Mineral, Rio de Janeiro, Brazil, 1978).

11. De Almeida, F. F. M., Hasui, Y., de Brito Neves, B. B. & Fuck, R. A. *Earth Sci. Rev.* **17**, 1-29 (1981).
12. Cordani, U. G. *et al. II Congr. Geol. Chileno, Arica*, **137-148** (1979).
13. Cordani, U. G. & de Brito Neves, B. B. *Rev. Bras. Geoc.* **12**, 78-88 (1982).
14. Tassinari, C. C. G. *Anais II Symp. Amaz. Manaus*, 439-446 (1984).
15. Kroonenberg, S. B. *Geologie Mijnb.* **61**, 325-333 (1982).
16. Priem, H. N. A. *et al. Geologie Mijnb.* **61**, 229-242 (1982).
17. Del'Arco, J. O. *et al. Simp. Geologia do Centro-Oeste Soc. Bras. Geol.*, 154-176 (1987).
18. Priem, H. N. A. *et al. Bull. geol. Soc. Am.* **82**, 1095-1102 (1971).
19. Lehmann, B. *Geol. Rdsch.* **67**, 270-278 (1978).
20. O'Connor, E. A. & Walde, D. H. G. *Abstr. 9th Geowissen. Lateinamerika Koll.* (Marburg, 1984).
21. Miller, H. J. *geol. Soc.* **141**, 885-892 (1984).
22. Gansser, A. *J. geol. Soc.* **129**, 93-131 (1973).
23. Windley, B. F. *The Evolving Continents* (Wiley, New York, 1977).
24. Kroner, A. in *Precambrian Plate Tectonics*, 57-90 (Elsevier, Amsterdam, 1981).
25. Williams, H. *Can. J. earth Sci.* **21**, 887-901 (1984).
26. *Departamento Nacional da Produção Mineral (Brazil) 1:1,000,000 Geol. Map Ser.*

Oceanic solitons, nutrient pulses and phytoplankton growth

P. M. Holligan*, R. D. Pingree† & G. T. Mardell†

* Marine Biological Association of the United Kingdom, Citadel Hill, Plymouth PL1 2PB, UK

† Institute of Oceanographic Sciences, Brook Road, Wormley, Godalming, Surrey GU8 5UB, UK

Phytoplankton growth in the sea is influenced by internal wave activity both through vertical displacements of the pycnocline, which cause fluctuations in the light environment for photosynthesis, particularly in the subsurface chlorophyll maximum^{1,2}, and through mixing processes associated with energy dissipation³. In shallow continental-shelf waters, mixing by internal waves can lead to pulses of nutrients in the nutrient-depleted surface layers⁴. In the deep oceans, increased nutrient fluxes resulting from internal wave mixing have also been postulated to explain anomalously high primary production values⁵, but this phenomenon has not been observed directly. Here we present the first evidence from a deep (4,000 m) oceanic environment for nutrient mixing by large soliton-like internal waves⁶⁻⁹, generated more than a day before at the shelf edge. The observations were made in the Bay of Biscay (Fig. 1a), where the level of nitrate is considered to be a major factor regulating the standing stock and productivity of phytoplankton in surface water during the summer months^{10,11}, as in other areas of the eastern North Atlantic Ocean¹².

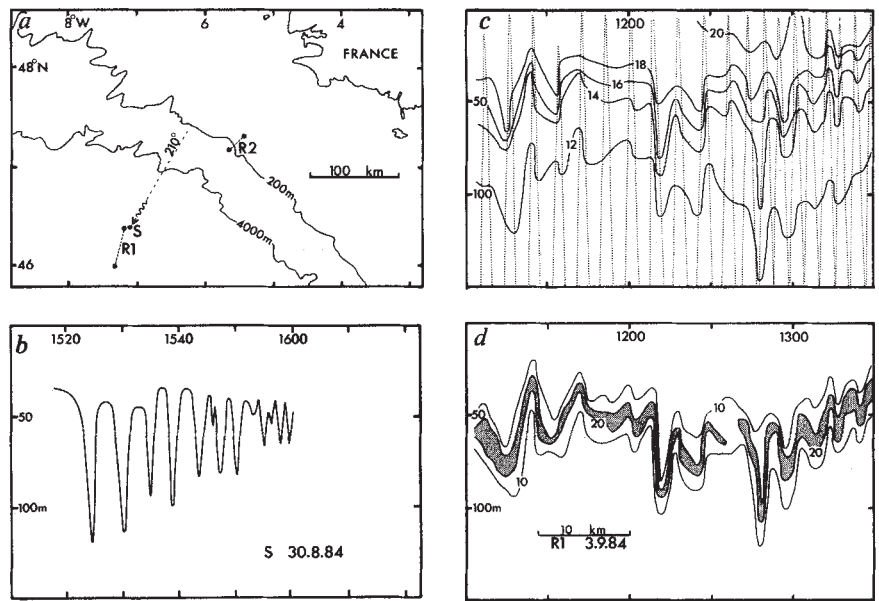
Earlier studies along the edge of the north-west European shelf showed that internal waves are generated in discrete groups or packets close to the 200-m contour and propagate on-shelf with on-shelf tidal streaming¹³. These are best developed at spring tides¹⁴. Synthetic Aperture Radar (SAR) images from the Seasat satellite indicate that internal waves also travel outwards from the shelf-break into the deeper waters of the Bay of Biscay¹³. Our investigations during large spring tides at the end of August 1984, confirmed the existence of these oceanic solitons, which were detected acoustically (Fig. 1b) as they propagated normally and away from (~210°) the shelf edge. Each packet of two to six large waves (followed by a train of smaller waves) was formed on a single tidal excursion. The peak-to-trough amplitude of these larger waves was 50-80 m, with a wavelength of 1-1.5 km and a phase speed of ~1.1 m s⁻¹. With calm winds (< 5 m s⁻¹), similar characteristics were found

at distances of up to 240 km from the shelf-break, corresponding to a travel time of more than two days. Throughout this time, the solitons could be seen visually and by radar as parallel bands of rough and smooth water of up to 10 km in length with associated lines of floating debris and scum. Records from a towed, undulating CTD-fluorometer system^{15,16} also show the presence of large internal waves (Fig. 1c, d). Within the limits of spatial resolution set by the sampling strategy, the variations in the depth of the subsurface chlorophyll maximum¹⁷ were consistent with the temperature (density) structure. These variations produce large fluctuations in ambient light conditions for the plant cells; for example, given a typical value for the extinction coefficient, k_d of 0.08 m⁻¹, the light intensity at the level of the chlorophyll maximum in Fig. 1d is estimated to range from ~6.0 to 0.03% of surface values.

Although the echo traces showed no overturning¹⁸ on a vertical scale of the internal wave amplitude, expendable bathythermograph (XBT) temperature profiles through individual waves exhibited occasional temperature inversions (Fig. 2). These temperature profiles are representative of the density profiles, as changes in salinity with respect to depth were both small (~0.05‰ from the surface down to 200 m) and destabilizing. The inversions cannot be interpreted in terms of interleaving between adjacent water masses, as horizontal salinity gradients above and below the pycnocline were also very weak (< 0.01‰ over 10 km). The most probable explanation, therefore, is active vertical mixing, at low Richardson Number conditions, due to the passage of the internal wave¹⁹. Areas of surface roughness, ~5 m across, in smooth water near the internal wave crests (Fig. 3) indicated small-scale turbulent events within the surface mixed layer. Local increases in surface nutrients (NO₃, NO₂, Si; Fig. 4a) were also detected as the wave packet passed the ship, similar to nutrient patches observed on the shelf in 1983 (P.M.H., R.D.P. and G.T.M., unpublished data), in association with a large amplitude internal wave packet propagating on the continental shelf¹⁴. The smoothing effects in the nutrient sampling system are such that the spatial extent of the nutrient patch (Fig. 4a) is unresolved and, as far as the instrumentation is concerned, is equivalent to a point source. The ephemeral nature of nutrient changes associated with internal waves was also a feature of measurements from the Southern Californian Bight⁴.

All crossings of the shelf break between 20 August and the period of strongest tides on 29-30 August had shown characteristic surface cooling and enhanced chlorophyll fluorescence¹⁷ but

Fig. 1 *a*, Bathymetric map of the northern Bay of Biscay. The wavy arrow indicates the direction of soliton propagation and points to the location (S) of the observations shown in Figs 1*b*, 2, 3 and 4*a*. R1 and R2 are the ship's tracks for the hydrographic records illustrated respectively in Figs 1*c*, *d* and 4*b*. *b*, Depth of the scattering layer (corresponding approximately to the 14 °C isotherm) against time (GMT) at a position ~140 km from the shelf-break (see *a*) as the RRS *Frederick Russell* steamed 020 °T at 5 knots on 30 August 1984. The internal waves are propagating from right to left (in the figure) in a direction 210° as determined from the ship's radar. A XBT section and temperature profiles through the leading wave of the soliton packet are shown in Fig. 2. A surface nutrient pulse was observed at 15:38 GMT (Fig. 4*a*). Water depth is 4,600 m. *c*, *d*, Undulator records for 11:00–13:30 GMT 3 September 1984, showing the distributions of temperature (°C) and chlorophyll fluorescence (arbitrary units) against depth (m). The fluorescence contours correspond to chlorophyll-*a* concentrations of about 0.4 and 0.8 mg m⁻³. The dotted lines in *c* indicate the path of the towed undulator system.



uniformly low (<0.2-μM) surface nitrate values. On 1–2 September, however, nitrate concentrations up to 1.5 μM were found at a location close to the 200-m contour (Fig. 4*b*), with values >1 μM over a distance of ~7 km normal to the shelf edge corresponding to a band of cold water. Throughout this period winds were weak (<5 m s⁻¹) and variable in direction, indicating that the increase in surface nutrients close to the shelf-break resulted from the cumulative and continuous effects of strong tidal streams and internal wave activity rather than from wind-induced upwelling²⁰.

During the summer months, the coupled effects of tides and wind promote vertical mixing within the relatively shallow (<200-m) water column on the continental shelf. By contrast, in the deep water environment of the Bay of Biscay, bottom mixing by tides is irrelevant and the effect of wind-mixing above a relatively deep pycnocline (~50 m, Fig. 1*c*) is much reduced so that vertical nutrient fluxes across the seasonal pycnocline

are relatively weak¹⁰. Furthermore, the subsurface chlorophyll maximum acts as a sink for nutrients mixed upwards from below the pycnocline²¹. Under these conditions phytoplankton growth in the surface waters tends to be restricted by the rate of nutrient supply from biological regeneration processes^{11,22,23}. The occurrence of the observed density inversions, surface nutrient patches and surface turbulence features in association with solitons indicates that mixing by internal waves is an important physical mechanism for maintaining upward fluxes of nutrients (another mechanism may be mesoscale eddies²⁴) as has been suggested⁵ for the North Pacific gyre. This is likely to be particularly significant at the level of the subsurface chlorophyll maximum in the pycnocline, where mixing by internal waves will produce more frequent nutrient pulses than are encountered at the sea surface.

Mixing by spring-tide solitons will be repeated at fortnightly intervals throughout the summer and influence phytoplankton

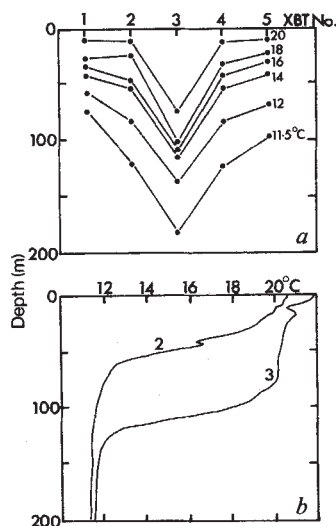


Fig. 2 *a*, Expendable bathythermograph (XBT) section across the first wave in the soliton packet illustrated in Fig. 1*b*. *b*, XBT profiles through the leading wave of the soliton packet showing temperature inversions.



Fig. 3 Sea surface state at position S (Fig. 1*a*) within soliton packet (Fig. 1*b*) at 15:00 GMT, 30 August 1984. Rough water, corresponding to the internal wave troughs, appears as dark bands towards the horizon. Surface layer turbulence is shown as disturbed patches, ~5 m across, in the foreground. Water depth is 4,600 m.

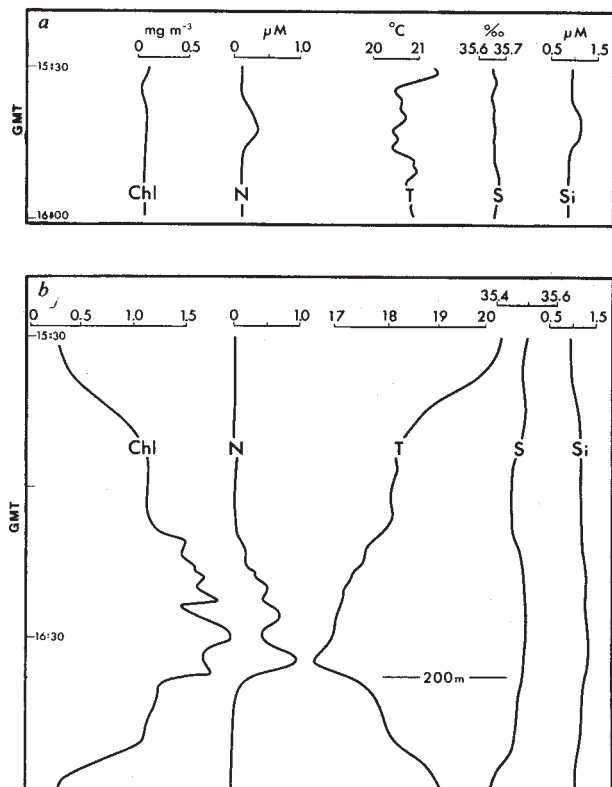


Fig. 4 Continuous surface (3.5-m) records of chlorophyll-*a* (Chl, mg m^{-3}), nitrate (N, μM), temperature (T, $^{\circ}\text{C}$), salinity (S, ‰) and silicate (Si, μM) for *a*, position S, 15:30–16:00 GMT, 30 August 1984, and *b*, track R2, 15:30–17:00 GMT, 2 September 1984, crossing the shelf-break at 16:37 GMT. The ship's positions are shown in Fig. 1*a* and in both cases the ship was heading in a southwesterly direction.

growth in the thermocline at great distances from the shelf-break. The horizontal extent of this effect will vary because of interaction with wind mixing and changes in the depth of the thermocline resulting from the generation of near inertial oscillations²⁵. At times of stronger winds, it is anticipated that mixing events due to instabilities associated with the passage of internal waves will occur more frequently and the nutrient pulses will then be more concentrated in the slope region.

We thank John Smithers, Bob Longmore and the Research Vessel Services Computing Group for assistance at sea.

Received 9 November 1984; accepted 28 January 1985.

- Haury, L. R., Wiebe, P. H., Orr, M. H. & Briscoe, M. G. *J. mar. Res.* **41**, 65–112 (1983).
- Kahru, M. *Mar. Ecol. Prog. Ser.* **10**, 111–117 (1983).
- Garrett, C. & Munk, W. *Deep-Sea Res.* **19**, 823–832 (1972).
- Cullen, J. J., Stewart, E., Renger, E., Eppley, R. W. & Winant, C. D. *J. mar. Res.* **41**, 239–262 (1983).
- McGowan, J. A. & Hayward, T. L. *Deep-Sea Res.* **25**, 771–793 (1978).
- Zabusky, N. J. & Krustal, M. D. *Phys. Rev. Lett.* **15**, 240–243 (1965).
- Osborne, A. R. & Burch, T. L. *Science* **208**, 451–460 (1980).
- Sandstrom, H. & Elliott, J. A. *J. geophys. Res.* **89**, 6415–6426 (1984).
- Briscoe, M. G. *Nature* **312**, 15 (1984).
- Tréguer, P., Le Corre, P. & Grall, J. R. *Deep-Sea Res.* **26A**, 1121–1152 (1979).
- Eppley, R. W., Renger, E. H. & Harrison, W. G. *Limnol. Oceanogr.* **24**, 483–494 (1979).
- Williams, R. & Robinson, G. A. *Bull. Mar. Ecol.* **7**, 115–121 (1973).
- Pingree, R. D. & Mardell, G. T. *Phil. Trans. R. Soc. A302*, 663–682 (1981).
- Pingree, R. D. & Mardell, G. T. *Prog. Oceanogr.* **14**, 431–441 (1985).
- Collins, D. S., Pollard, R. T. & Suchen, P. V. *Inst. Oceanogr. Sci. Rep.* **148**, 1–75 (1983).
- Fasham, M. J. R., Pugh, P. R., Griffiths, D. & Wheaton, J. E. G. *Proc. Inst. Electr. Radio Engng Lond.*, 49–58 (1981).
- Pingree, R. D., Mardell, G. T., Holligan, P. M., Griffiths, D. K. & Smithers, J. *Contin. Shelf Res.* **1**, 99–116 (1982).
- Haury, L. R., Briscoe, M. G. & Orr, M. H. *Nature* **278**, 312–317 (1979).
- Woods, J. D. & Wiley, R. L. *Deep-Sea Res.* **19**, 87–121 (1977).
- Heaps, N. S. *Oceanol. Acta* **3**, 449–454 (1980).
- Pingree, R. D., Holligan, P. M. & Head, R. N. *Nature* **265**, 266–269 (1977).
- King, F. D. & Devol, A. H. *Limnol. Oceanogr.* **24**, 645–651 (1979).
- Holligan, P. M., Williams, P. J. LeB., Purdie, D. & Harris, R. P. *Mar. Ecol. Prog. Ser.* **17**, 201–213 (1984).
- Madelain, F. & Kerut, E. G. *Oceanol. Acta* **1**, 159–168 (1978).
- Mazé, R. thesis, Univ. Bretagne Occidentale (1983).

Lake acidification in Galloway: a palaeoecological test of competing hypotheses

Richard W. Battarbee*, Roger J. Flower*, Anthony C. Stevenson* & Brian Rippey†

* Palaeoecology Research Unit, Department of Geography, University College London, 26 Bedford Way, London WC1H 0AP, UK
† Limnology Laboratory, The University of Ulster, Traad Point, Magherafelt, Co. Derry BT45 6LR, UK

Possible causes of lake acidification in Britain include acid precipitation, heathland regeneration, afforestation and post-glacial natural acidification (long-term change). In Galloway, south-west Scotland, several lakes with non-afforested catchments have been acidified by approximately 1 pH unit since about 1840^{1,2}, which clearly precludes long-term change and afforestation as causes of the present acidity, but does not discriminate between the possible effects of heathland regeneration and acid precipitation; it could be argued that a decline in upland farming in this area led to an increase in acid heathland communities capable of promoting lake acidification during this period of time. Here we use pollen analysis to show that there is no evidence for an increase in *Calluna vulgaris*, the most important heathland species, over the past 200 yr or so, and we substantiate the acid precipitation hypothesis by demonstrating substantial increases of the heavy metals Pb, Cu and Zn in the sediment since about AD 1800.

Surface water acidification has been variously ascribed to the effects of acid precipitation³, land-use change⁴, afforestation^{5,6} and long-term change⁷, the importance of which can be assessed by palaeoecological techniques. We consider the last two of these processes first.

Long-term acidification is well known, having been recognized in Scandinavia^{8–11}, Britain^{12–15} and North America (ref. 16 and J. Ford, unpublished results). In Britain it has been suggested that this process is the cause of contemporary acidity in upland tarns of the English Lake District and that certain lakes were so acidic by 1800 that no further change can have occurred since then⁷. Although this supposition has not yet been examined in the context of the Lake District, it is clear that the present acidity of lakes with granitic catchments in Galloway cannot be explained in this way; diatom analysis indicates that rapid acidification of these lakes has occurred since about 1840, after long periods of stability^{1,2}.

Recent afforestation, although responsible for accelerated soil erosion¹⁷ and other undesirable ecological changes, is also of little significance as lakes with non-afforested catchments were

Table 1 Physical and chemical characteristics for Loch Enoch

Altitude (m)	493	
Lake area (ha)	50	
Catchment area (ha)	186	
Catchment: lake area	3.7	
Maximum depth (m)	~36	
Temperature ($^{\circ}\text{C}$)	7.0 (4.4)	
Conductivity ($\mu\text{S}_{18} \text{ cm}^{-1}$)	30.0 (8.0)	37*
pH (range)	4.4–4.7	4.5*
H^{+} ($\mu\text{eq l}^{-1}$)	28.9 (6.8)	28
Na^{+} ($\mu\text{eq l}^{-1}$)	58.3 (24.4)	126
K^{+} ($\mu\text{eq l}^{-1}$)	6.7 (4.6)	6
Ca^{2+} ($\mu\text{eq l}^{-1}$)	10.5 (3.5)	21
Mg^{2+} ($\mu\text{eq l}^{-1}$)	16.5 (7.2)	37
Al ($\mu\text{eq l}^{-1}$)	10.0 (4.4)	16
Cl ($\mu\text{eq l}^{-1}$)	100.9 (18.0)	146
SO_4^{2-} ($\mu\text{eq l}^{-1}$)	44.5 (12.9)	89

Numbers in parentheses indicate standard deviations for the mean values calculated from eight measurements made between November 1981 and November 1982. The catchment area excludes lake area.

* Data in the last column are from ref. 33.

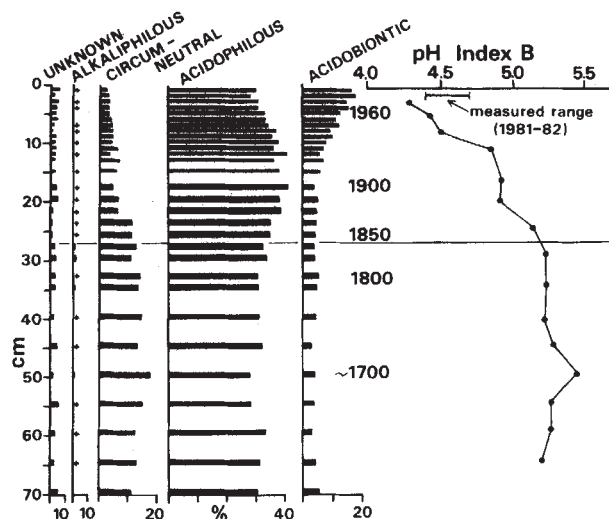


Fig. 1 Summary diatom diagram from Loch Enoch showing changes in the proportions of the various pH groups through time. Dominant taxa are as follows: (1) circumneutral, *Anomoeoneis vitrea* (Grun.) Ross; (2) acidophilous, *Eunotia veneris* (Kütz.) O. Müll., *E. alpina* (Naeg.) Hust., *Tabellaria flocculosa* (Roth.) Kütz., *Frustulia rhomboides* v. *saxonica* (Rabh.) de Toni; (3) acidobiontic, *T. quadriseptata* Knudson, *T. binalis* (Ehr.) Grun., *Navicula höfleri* Chlonoky. Dates are based on ^{210}Pb measurements¹⁷, and pH is reconstructed using index B¹¹. Horizontal line signifies point of change.

acidified, and those with afforested catchments began to be acidified, in the late nineteenth and early twentieth century, well before the beginning of afforestation¹.

A hypothesis involving change in land use has been proposed to explain recent lake acidification in Norway^{4,18}. It maintains that acidity generated by soil processes is substantially greater than that received from atmospheric deposition and that an increase in the formation of acid humus in soils as a result of a decline in upland agriculture is likely to be of more significance than an increase in the acidity of precipitation.

In Galloway it may be expected that a similar decline in grazing would lead to the greater dominance of *C. vulgaris* in particular, and to a regeneration of heathland communities in general¹⁹. This hypothesis would be rejected if it could be shown that such a land-use change was absent or was inadequate to explain the observed acidification of the lakes concerned, and we would then need to evaluate the acid precipitation hypothesis.

To carry out these tests we have used diatom analysis, pollen analysis and trace metal analysis of a dated lake sediment core from Loch Enoch, a 50-hectare, 36-m deep multi-basin lake situated at an altitude of 493 m on the Merrick in the Rhinns of Kells region of Galloway. The catchment is non-afforested and the vegetation is dominated by *Molinia caerulea* and *C. vulgaris*.

We obtained a sediment core from the centre of the south-east basin of the lake and determined a linear sediment accumulation rate of $\sim 1 \text{ mm yr}^{-1}$ using ^{210}Pb analysis¹⁷. Diatom analysis shows the onset of recent acidification at $\sim 30 \text{ cm}$, dated to about 1840 (Fig. 1). The index B pH reconstruction¹¹ gives a c.1840 pH of 5.2 and a present pH of 4.3, slightly more acidic than the measured range of pH values (see Table 1). The strong increase in acidobiontic diatoms registered in the diagram (Fig. 1) include *Tabellaria binalis* (Ehr.) Grun. and *Tabellaria quadriseptata* Knudson, species that typically increase in a similar way at other sites in the region^{1,20}.

If acidification was caused by a change in land use in the catchment of the lake, in accordance with the above hypothesis, we would expect historical records to show a decline in grazing and consequent regeneration and expansion of heathland on approximately the same timescale. As records of sheep numbers

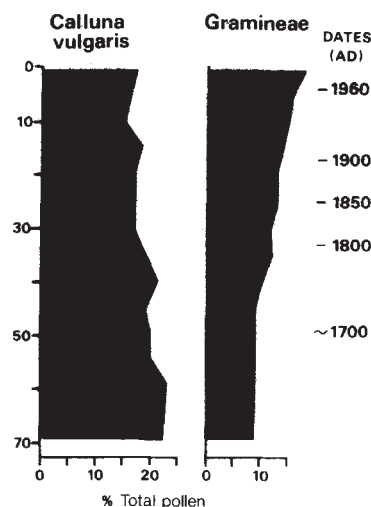


Fig. 2 Proportions of *C. vulgaris* and Gramineae pollen from the Loch Enoch sediment core.

over the past 150 yr are available only for large administrative regions²¹, it is more appropriate to test this prediction using pollen analysis of the lake sediment core itself. This has been performed using standard procedures²² on samples selected at 5-cm intervals from the top of the core. The full pollen diagram shows very little change over the past 200–300 yr, except in the relative proportions of *C. vulgaris* and Gramineae, the taxa of greatest interest (Fig. 2). However, rather than an increase in *C. vulgaris*, as would be expected were the land-use hypothesis valid at these sites, there is a gradual decrease in this species and a reciprocal expansion of Gramineae; similar results have been obtained from the analysis of cores from other lakes in the area both in terms of percentage and flux change (unpublished results). These results are not unexpected as they agree with contemporary observations that all the non-afforested lake catchments in this area are grazed and repeatedly burnt over, a process that in the west of Scotland favours *M. caerulea* and keeps *C. vulgaris* young and palatable for both cattle and sheep²³.

Having rejected three hypotheses for the recent acidification of Loch Enoch, we retain only the acid precipitation hypothesis. This hypothesis would be questionable if we could not show that Loch Enoch and other similar Galloway lakes have received atmospheric pollutants that may be associated with industrial emissions. We therefore analysed the trace metal content of the sediments (Fig. 3).

Total sedimentary Zn, Cu and Pb concentrations were determined by flame atomic absorption after $\text{HF}/\text{HNO}_3/\text{HClO}_4$

Table 2 Background sedimentary concentrations, fluxes and recent fluxes of Zn, Cu and Pb in a sediment core from Loch Enoch

	Zn	Cu	Pb
Background concentration, $\mu\text{g g}^{-1}$	25.4	6.2	74.6
Standard deviation (less than 32 cm depth, $n = 12$)	7.4	4.6	23.3
Background sedimentary flux, $\text{mg m}^{-2} \text{yr}^{-1}$ (average value for 32–61 cm)	6.1	1.5	17.8
Recent sedimentary flux, $\text{mg m}^{-2} \text{yr}^{-1}$ (average value for 0–10 cm)	35.5	8.3	67.0
Anthropogenic sedimentary flux, $\text{mg m}^{-2} \text{yr}^{-1}$ (recent, background flux)	29.4 (83)	6.8 (82)	49.2 (73)

Calculations were made using trace metal concentrations (Fig. 3) and sediment wet density, dry weight and accumulation rate (ref. 21). Numbers in parentheses indicate % anthropogenic flux of total metal influx.

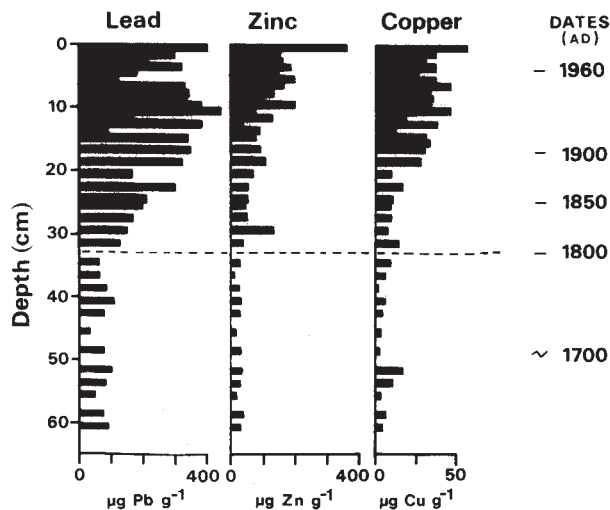


Fig. 3 Concentrations of Pb, Zn and Cu from the Loch Enoch sediment core. Dotted line signifies point of change.

digestion, the precision of the results are, respectively, 4.7, 0.9 and $3.8 \mu\text{g g}^{-1}$ ($n = 14$). The background concentrations (Table 2) show that there are no geochemical anomalies in the catchment. The slightly lower Cu and Zn and somewhat higher Pb concentrations, compared with the mean for freshwater sediments²⁴, are a result of the granites in the catchment²⁵. The increased concentrations (Fig. 3) and sedimentary fluxes (Table 1) can be accounted for by increased deposition from the atmosphere²⁶, a situation common in remote and rural lakes²⁷⁻²⁹. It has been shown that atmospheric contamination of lake waters by trace metals is correlated with acidification^{30,31}, and that contamination of sediments is correlated with sedimentary evidence of acidification^{26,27,32}. This latter relationship holds true for Loch Enoch and all these relationships are consistent with expectations derived from the acid precipitation hypothesis.

We cannot prove that an increase in acid deposition was responsible for the acidification of Loch Enoch and similar lakes in Galloway, but we have shown that alternative hypotheses, as presently formulated, are inadequate. Land-use change may play a part in other areas, but at present it is clearly not applicable to the Galloway sites. For this hypothesis to be tested by palaeoecological means, examination of an acidified site in an area of low acid deposition, where heathland regeneration has occurred concurrently, is required.

We thank the Central Electricity Generating Board for funding this work, CERL, Leatherhead, for carrying out chemical analyses of Loch Enoch water samples, Stuart Phethean for help with fieldwork, Kevin Odell for radiometric data, Peter Appleby for ^{210}Pb computations, and Richard Skeffington and Gwyneth Howells for comments on the manuscript.

Received 6 December 1984; accepted 9 January 1985.

1. Flower, R. J. & Battarbee, R. W. *Nature* **305**, 130-133 (1983).
2. Battarbee, R. W. *Phil. Trans. R. Soc. B* **305**, 451-477 (1984).
3. Jensen, K. W. & Snekvik, E. *Ambio* **1**, 223-225 (1972).
4. Rosenquist, I. T. *Sci. tot. Envir.* **10**, 39-49 (1978).
5. Harriman, R. & Morrison, B. R. S. *Hydrobiologia* **88**, 251-263 (1984).
6. Nilsson, S. I., Miller, H. G. & Miller, J. D. *Oikos* **39**, 40-49 (1982).
7. Pennington, W. A. *Rep. Freshwat. biol. Ass.* 28-46 (1984).
8. Quennerstedt, N. *Acta phytogeogr. suec.* **36**, 1-208 (1955).
9. Digerfeldt, G. *Folia limnol. scand.* **16**, 1-104 (1972).
10. Renberg, I. *Early Norrland* **9**, 113-160 (1976).
11. Renberg, I. & Hellberg, T. *Ambio* **11**, 30-33 (1982).
12. Round, F. E. *New Phytol.* **56**, 98-126 (1957).
13. Mackereith, F. J. H. *Proc. R. Soc. B* **161**, 295-309 (1965).
14. Pennington, W., Haworth, E. Y., Bonny, A. P. & Lishman, J. P. *Phil. Trans. R. Soc. B* **264**, 191-294 (1972).
15. Evans, G. H. & Walker, R. *New Phytol.* **78**, 221-236 (1977).
16. Engstrom, D. R. & Wright, H. E. in *Lake Sediments and Environmental History* (eds Haworth, E. Y. & Lund, J. W. G.) (Leicester University Press, 1984).
17. Battarbee, R. W., Appleby, P. G., Odell, K. & Flower, R. J. *Earth Surf. Processes* (in the press).
18. Krug, E. E. & Frink, C. R. *Science* **221**, 520-525 (1983).
19. Gimingham, C. H. *J. Ecol.* **48**, 455-483 (1960).
20. Flower, R. J. & Battarbee, R. W. *Br. phycol. Bull.* **21**, (in the press).
21. Flower, R. J. & Battarbee, R. W. *Working Pap. No. 6* (Palaeoecology Research Unit, University College London, 1983).

22. Moore, P. D. & Webb, J. A. *An Illustrated Guide to Pollen Analysis* (Hodder & Stoughton, Dunton Green, 1978).
23. McVean, D. N. & Lockie, J. D. *Ecology and Land Use in Upland Scotland* (Edinburgh University Press, 1969).
24. Forstner, U. *Arch. Hydrobiol.* **50**, 172-191 (1977).
25. Mason, B. & Moore, C. B. *Principles of Geochemistry* (Wiley, New York, 1982).
26. Galloway, J. N., Thornton, J. D., Norton, S. A., Volchok, H. L. & McLean, R. A. N. *Atmos. Envir.* **16**, 1677-1700 (1982).
27. Davis, R. B., Norton, S. A., Brakke, D. F., Berge, F. & Hess, C. T. in *Ecological Impact of Acid Precipitation* (eds Drablos, D. & Tolland, A.) (SNSF project, Oslo, 1980).
28. Rippey, B., Murphy, R. J. & Kyle, S. W. *Envir. Sci. Technol.* **16**, 23-30 (1982).
29. Wong, H. K. T., Nragu, J. O. & Coker, R. D. *Chem. Geol.* **44**, 187-201 (1984).
30. Henriksen, A. & Wright, R. F. *Wat. Res.* **12**, 101-112 (1978).
31. Wright, R. F. & Henriksen, A. *Limnol. Oceanogr.* **23**, 487-498 (1978).
32. Holdren, G. R., Brunelle, T. M., Matisoff, G. & Wahlen, M. *Nature* **311**, 245-248 (1984).
33. Wright, R. F. & Henriksen, A. *Regional Surv. Lakes Streams Southwestern Scotland* (SNSF project IR 72/80, 1980).

Sediment and pollen evidence for an early to mid-Holocene humid period in the eastern Sahara

J. C. Ritchie*, C. H. Eyles† & C. V. Haynes‡

* Scarborough Campus and †Department of Geology, University of Toronto, Scarborough, Ontario, Canada M1C 1A4

‡ Departments of Anthropology and Geosciences, University of Arizona, Tucson, Arizona 85721, USA

A major problem in the study of Holocene palaeoenvironments of the arid and wind-deflated Sahara is the low preservation potential of sediments from which a record of past climatic change can be established. Several indirect and imprecisely dated pieces of evidence have suggested humid episodes in the Holocene that may have supported more productive ecosystems and denser human populations than today. Searches for reliable proxy data on past climates, however, have failed to yield satisfactory results. We report here the discovery of buried lake muds in north-west Sudan, in the hyperarid core of the Eastern Sahara, which yield sedimentological and palynological data clearly interpretable as recording an early to mid-Holocene humid episode that supported a relatively-deep stratified lake surrounded by tropical savanna woodland vegetation.

The site lies in the Oyo Depression in NW Sudan at $19^{\circ}16'N$ and $26^{\circ}11'E$, 510 ± 20 m above sea level (Fig. 1). The depression is surrounded by bedrock and contains Holocene lacustrine sediments buried by variable depths of dune sand¹. It lies in the hyperarid core of the Sahara, with an estimated mean annual rainfall of ~ 5 mm (ref. 2), northerly winds with mean daily average velocity of $2-20 \text{ km h}^{-1}$, daily average relative humidity of 20-60% and estimated evaporation rates of $\sim 5 \text{ m yr}^{-1}$ (ref. 3). The area is in the zone of absolute desert vegetation whose southern limit is ~ 500 km south of the site⁴ (Fig. 1). Vegetation is confined to oases, supported by artesian water, and to wadi channels where episodic rainstorms produce transient communities of annuals and herbaceous perennials⁴.

Table 1 Radiocarbon dates from the Oyo sediments

Site and depth from surface (cm)	Material analysed	Radiocarbon laboratory number	Age (yr BP)
Hole-6, 170-180	Algal sapropel	SMU-1232	$4,920 \pm 200$
Hole-6, 270-280	Algal sapropel	SMU-1233	$5,880 \pm 80$
Hole-6, 310-320	Algal sapropel	SMU-1231	$6,100 \pm 80$
Hole-6, 460-470	Algal sapropel	SMU-1230	$8,490 \pm 90$
Hole-6, 460-470	Charcoal	AA-253	$8,030 \pm 280$
Auger-1, 460	Charcoal	A-2826	$8,900 \pm 120$
Auger-1, 180	Algal sapropel	A-2820	$4,590 \pm 140$
Well-2, 200	Charcoal	A-2951	$8,720 \pm 170$

The auger-1 site samples were collected from the basin sediments 150 m north-west of the hole-6 pit; the well-2 charcoal sample was recovered from the base of the Zone I sediments exposed in a hand-dug well 260 m west of the hole-6 pit, at a depth of 200 cm from the surface, near the margin of the basin. The analyses were conducted at the Radiocarbon Laboratory of Southern Methodist University, Texas (SMU) and at the Arizona National Science Foundation Regional Facility for Accelerator Mass Spectrometry, Tucson, Arizona (A).

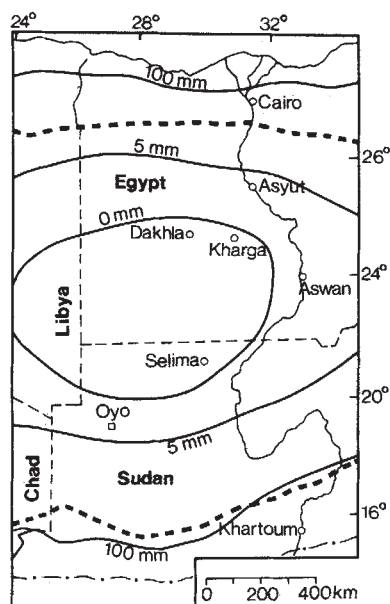


Fig. 1 Map of the Eastern Sahara region showing the location of the Oyo site, the 0, 5 and 100-mm mean annual precipitation limits (after Dubief²), the northern and southern limits of absolute desert (---) and the northern limit of the savanna (- · - · -) (both after White⁴).

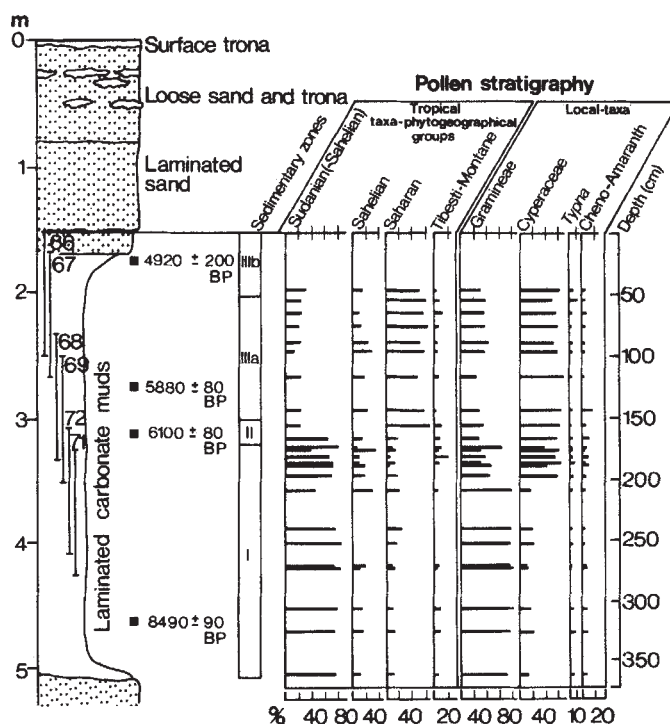


Fig. 2 A summary diagram showing the sediment stratigraphy, positions of the hole-6 1-m tray samples (numbered), positions and values of the radiocarbon age estimates and the pollen stratigraphy of the main groups. The phytogeographical pollen groups are expressed as percentages of the tropical taxa and the sum of the local taxa is the pollen total for the 'local' percentages.

The sample site is in a small basin, $\sim 1.0 \times 0.5$ km in size, in a 2-km wide depression open to the south-west and bounded by sandstone escarpments. The sediments were discovered in 1980 and sampled in 1982 and 1983, by hand augering and by digging a 5-m deep pit (hole-6 pit) in the centre of the basin¹. Undisturbed overlapping 1-m lengths of sediment were recovered from the pit face in aluminium trays each 5 cm in width and depth. The lowest levels were collected by discontinuous bulk sampling because groundwater made tray sampling impossible. Four levels in hole-6 pit and three from nearby auger holes were dated by radiocarbon analysis (Fig. 2, Table 1). The radiocarbon dates of algal sapropel can be considered to be maximum values because of the possibility of error due to older carbonates. The charcoal from the 460–470-cm level in hole-6, dated by the tandem accelerator mass spectrometer (TAMS), indicates a potential discrepancy, but the large standard deviation precludes any firm conclusions about the magnitude of the carbonate error.

Sedimentological analysis suggested that the sequence can be divided into three major zones⁵. Zone 1 consists predominantly of finely laminated (0.1–1.0-mm) carbonate muds interbedded with sequences of pure white and grey laminae, dark organic bands and bioturbated horizons. The finely laminated muds are composed of green/brown and cream microcrystalline dolomite with trace amounts of clay minerals. Annual deposition of these couplets is suggested by comparing sedimentation rates estimated from ¹⁴C dates (Fig. 2; 1 cm every 11–15 yr) with the counted average of 13 couplets cm^{-1} . Units of pure white and grey laminations consist predominantly of calcite with minor amounts of quartz and dolomite and are always associated with dark organic-rich bands composed of algal remains (Fig. 3).

Zone I sediments indicate carbonate deposition in a stratified lake with stagnant bottom waters^{6–8}. The predominance of dolomite with an olive-green colour and absence of burrowing organisms through most of this zone suggests reducing conditions, characteristic of arid perennial lakes receiving a restricted seasonal supply of fresh water⁸. The association of white and grey laminations, organic bands and bioturbated horizons (Fig. 3) suggests that episodic increased inflow of calcium-bearing freshwater into the basin and/or temperature fluctuations are responsible for triggering calcite precipitation, algal blooms and weakening of the halocline.

Zone II sediments consist of laminated carbonate muds similar to the green/brown and cream laminations of zone I but with irregularities in thickness, abundant soft sediment deformation structures and evidence of downslope resedimentation and slumping of sediment blocks. Such increased sediment instability may have been caused by lowered lake levels.

Zone III consists of a coarsening upwards sequence of highly deformed muds and sands. The deformed and crudely bedded dolomitic muds of zone IIIa contain significant amounts of windblown sand, which may indicate shallowing of the lake body. Final desiccation is indicated by the crudely bedded and bleached aeolian sands of zone IIIb (Fig. 2).

So far, 55 pollen taxa have been recorded (by J.C.R.) in the sediments, belonging to the following phytogeographical groupings^{9–12}: (1) Sudano-Sahelian taxa, found today in the tropical savannas of north-central Sudan and adjacent territories of Africa; (2) Sahelo-Saharan taxa with modern distribution in the thorn scrub and herbaceous desert belt of North Africa; (3) Saharan taxa, today confined to the Sahara and adjacent Arabian deserts. (These tropical taxa ((1)–(3) above) are listed in Table 2.) (4) Tibesti-Montane elements, so designated because the nearest pollen source is the Tibesti Massif, represented here by *Artemisia*, *Ephedra* (two types) and *Erica arborea*; (5) Mediterranean taxa, represented by rare occurrences in a few levels of *Betula*, *Picea*, *Pinus* and *Quercus*; and (6) a group of taxa of uncertain geographical affinity (Gramineae, Cyperaceae, *Typha*, Compositae-Asteraceae, Compositae-Fenestratae, Chenopodiaceae-Amaranthaceae, Leguminosae and *Myriophyllum*). Many of the tropical taxa are plants with low pollen productivity (for example *Piliostigma*, *Grewia*) and, in addition, several produce large ($>60\text{-}\mu\text{m}$) pollen grains with low dispersal capacity (*Acacia*, *Abutilon*, *Delonix*, *Hibiscus*, *Commicarpus*, *Grewia tenax* and *Pavonia*); thus, although they occur in low concentrations (100–500 grains ml^{-1}), their presence, in a state of excellent preservation, suggests that the source plants were close to, or in, the sedimentary basin.

Table 2 Tropical pollen taxa recovered from the sediments, grouped according to their phytogeographical affinities

Sudanian (-Sahelian)	Sahelian (Sudanian)	Saharan
<i>Acacia</i> cf. <i>sieberana</i>	<i>Abutilon</i>	<i>Aerva</i>
<i>Borreria radiata</i>	<i>Acacia</i> cf. <i>nilotica</i>	<i>Cleome</i>
<i>Boscia</i>	<i>Acacia</i> cf. <i>senegal</i>	<i>Maerua</i>
<i>Cadaba</i>	<i>Acacia</i> cf. <i>seyal</i>	<i>Salvadora</i>
<i>Celtis integrifolia</i>	<i>Acacia</i> cf. <i>raddiana</i>	<i>Tamarix</i>
<i>Chlorophytum</i>	<i>Atractylis</i>	
<i>Commicarpus</i>	<i>Balanites</i>	
<i>Commiphora</i>	<i>Blepharis</i>	
<i>Delonix regia</i>	<i>Borreria</i> cf. <i>chaetocephala</i>	
<i>Grewia</i> cf. <i>tenax</i>	<i>Capparis</i>	
<i>Hibiscus</i> (2 taxa)	<i>Cassia</i>	
<i>Lannea</i>	<i>Chroxophora</i>	
<i>Pavonia</i>	Combretaceae	
<i>Piliostigma</i> cf. <i>thoningii</i>	<i>Corchorus</i>	
<i>Piliostigma</i> cf. <i>reticulatum</i>	<i>Ottelia</i>	
<i>Ruellia</i>	<i>Phyllanthus maderaspatensis</i>	
<i>Zizyphus</i>	<i>Polygala</i> cf. <i>erioptera</i>	

Gramineae and Cyperaceae, the dominant taxa in all samples, have pollen concentrations between 10,000 and 60,000 grains ml^{-1} , indicating both a regional and local component.

The pollen stratigraphy shows a close correlation with the sediment zones (Fig. 2). The lower levels, 360–170 cm, exhibit a predominance, among the tropical taxa, of Sudano-Sahelian elements of which *Grewia tenax* and *Piliostigma*, both tree taxa characteristic of the modern deciduous savanna zone, are notable. Zones II and III of the sediment column are characterized by an abrupt increase in Sahelian and Saharan elements, typical of modern Sahelian savannas and thorn scrub vegetation. A significant change in the proportions of Gramineae and Cyperaceae occurs at approximately the Zone I/II boundary (Fig. 2). This change is difficult to interpret, but the relative increase in Cyperaceae could have been caused by a lowered lake level producing a larger marginal marshy zone; alternatively, part of the increase may have been the result of an expansion of such important dune taxa as *Cyperus conglomeratus*.

The sediment, pollen and radiocarbon data from this site indicate (1) that between 8,500 and 6,100 yr BP the Oyo depression supported a relatively deep stratified lake with stagnant bottom water and annual inputs of fresh water, surrounded by a continuous deciduous savanna vegetation, similar to that found today ~500 km to the south; (2) that at about 6,000 yr BP a reduction in precipitation and/or an increase in evaporation initiated a shallowing of the lake; and (3) that between 6,000 and 4,500 yr BP the lake became still shallower and the tropical Sudano-Sahelian savannas were replaced by *Acacia*-thorn savanna and scrub-grassland. Finally, at 4,500 yr BP the lake basin dried out and was covered subsequently by aeolian sediments, whereas the vegetation, in the form of desert scrub-grasslands, disappeared from all habitats except at oases and wadis.

The Holocene lake at Oyo was probably groundwater supported, as at Merga Lake, 40 km to the south-east, today¹³. Recent isotope investigations of shallow groundwater in the region support a model of direct recharge^{14–16} through the oases depressions, as opposed to subsurface flow over great distances, such as from the Enedi highlands in the case of Oyo.

The palaeoclimates suggested by the above conclusions are: for Zone I, a humid tropical climate with annual monsoonal rainfall of at least 400 mm; for Zones II and III, a progressive increase in aridity with annual precipitation declining from 300 mm at 6,000 yr BP to <100 mm at 4,500 yr BP. These reconstructions corroborate previous conclusions based on evidence from laminated lake sediments^{17–20}, East Mediterranean sapropels²¹, geomorphology and archaeology^{22,23} and Nile River flood history²⁴. Our results provide the first conclusive demonstration of vegetation and climate change in the early to mid-Holocene of the eastern Sahara. They agree with tentative conclusions of Maley^{25–27} for the distant Lake Chad record,

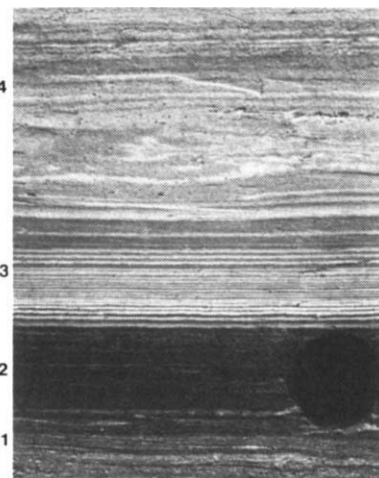


Fig. 3 Segment of Zone I (188–193 cm) sediments, showing: (1) finely laminated green/cream dolomite-rich muds; (2) finely laminated dark organic unit; (3) pure white/grey laminae, thinning upwards; (4) bioturbated laminated muds. Sample hole is 1 cm in diameter.

with the general Holocene lake level trends for north-east Africa^{28,29} and with changes in the position of the African monsoon predicted on the basis of Milankovitch orbital forcing factors^{30,31}. Detailed sediment, radiocarbon and pollen analyses of the Oyo deposits will be reported elsewhere.

The fieldwork was supported by funds (to C.V.H.) from the NSF (Grant EAR-8312651), the National Geographical Society and the Smithsonian Institute Foreign Currency Program; C.V.H. thanks Dr Bahay Isawi of the Geological Survey of Egypt for his cooperation, the Department of Geological and Mineral Resources of the Sudan for field support and Donald L. Johnson, John W. Olsen and Ahmed Swedan for help in the field; the TAMS radiocarbon dates are provided by the Arizona-NSF Regional Facility for Accelerator Mass Spectrometry. We thank the Natural Sciences and Engineering Research Council of Canada for a Postgraduate Scholarship (C.H.E.) and an operating grant (A6320) (J.C.R.) and Mme Daneille Duzer and Dr Jean Maley for help with the palynological analyses while J.C.R. was at the Laboratoire de palynologie, CNRS, Montpellier, France (visit arranged by Mme M. Van Campo). We also thank Keith Bennett, Les Cwynar, Nick Eyles and Tim Barrett for helpful comments, and K. A. Hadden for technical assistance.

Received 30 October 1984; accepted 16 January 1985.

- Haynes, C. V. *Natn. Geogr. Soc. Res. Rep.* 16 (in the press).
- Dubief, J. *Mém. Inst. Rech. Sah. Univ. Alger* 2, 1–275 (1963).
- Haynes, C. V. *Natn. Geogr. Soc. Res. Rep.* 15, 257–293 (1983).
- White, F. *The Vegetation of Africa* (UNESCO, Paris, 1983).
- Eyles, C. H., Haynes, C. V. & Ritchie, J. C. *Geol. Soc. Am. Abstr.* 16, 505 (1984).
- Wasson, R. J., Smith, G. I. & Agrawal, D. P. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* 46, 345–372 (1984).
- Eugster, H. P. & Hardie, L. A. in *Lakes: Chemistry, Geology Physics* (ed. Lerman, A.) 237–293 (Springer, New York, 1978).
- Smith, G. I. *US Geol. Surv. Prof. Pap.* 1043, 130 (1979).
- Wickens, G. E. *The Flora of Jebel Marra* (HM Stationery Office, London, 1976).
- Lebrun, J.-P. *Eléments pour un Atlas des Plantes Vasculaires de l'Afrique sèche* Vol. 1 (Inst. d'élevage et de médecine vétérinaire des pays tropicaux, Maisons Alfort, 1977).
- Lebrun, J.-P. *Eléments pour un Atlas des Plantes Vasculaires de l'Afrique sèche* Vol. 2 (Inst. d'élevage et de médecine vétérinaire des pays tropicaux, Maisons Alfort, 1979).
- Lebrun, J.-P. *Catalogue des Plantes Vasculaires du Niger* (Inst. d'élevage et de médecine vétérinaire des pays tropicaux, Maisons Alfort, 1976).
- Haynes, C. V., Mehninger, P. J. & Zaghloul, S. A. *Geogr. J.* 145, 437–445 (1979).
- Haynes, C. V. & Haas, H. *Radiocarbon* 22, 705–717 (1980).
- Muller, A. B. & Haynes, C. V. in *Isotope Hydrology in Water Resources Development* (IAEA, Vienna, 1984).
- Thoreweilhe, U., Schneider, M. & Sonntag, C. *Berliner Geowissenschaftliche Abhandlungen* 50, 209–216 (1984).
- Haynes, C. V. *Geol. Soc. Am. Abstr.* 14, 511 (1982).
- Mehninger, P. J. *Jr Geol. Soc. Am. Abstr.* 14, 512 (1982).
- Baumhauer, R. & Schulz, E. *Palaeoecol. Africa* 16, 283–289 (1984).
- Gabriel, B. & Kropelin, S. *Palaeoecol. Africa* 16, 295–299 (1984).
- Rosignol-Strick, M. *Nature* 303, 46–49 (1983).
- Haynes, C. V. *Science* 217, 629–633 (1982).

23. Haynes, C. V. in *Prehistory of the Eastern Sahara* (ed. Wendorf, F. & Schild, R.) 353-371 (Academic, New York, 1980).
24. Adamson, D. A., Gasse, F., Street, F. A. & Williams, M. A. J. *Nature* **287**, 50-55 (1980).
25. Maley, J. *Etudes palynologiques dans le bassin du Tchad et paléoclimatologie de l'Afrique nord-tropicale de 30 000 ans à l'époque actuelle* (Travaux et Documents de L'ORSTOM 129, Paris 1981).
26. Maley, J. *Nature* **269**, 573-577 (1977).
27. Maley, J. *Bothalia* **14**, 377-389 (1983).
28. Street, F. A. & Grove, A. T. *Nature* **261**, 385-390 (1976).
29. Street, F. A. & Roberts, N. in *Variations in the Global Water Budget* (ed. Street, F. A. et al.) 331-345 (Reidel, Dordrecht, 1983).
30. Kutzbach, J. E. *Quat. Res.* **14**, 210-223 (1980).
31. Kutzbach, J. E. & Otto-Bliesner, B. L. *J. Atmos. Sci.* **39**, 1177-1188 (1982).

A terrestrial fauna from the Scottish Lower Carboniferous

S. P. Wood*, A. L. Panchen† & T. R. Smithson†

* 'Mr Wood's Fossils', Unit 8, Abbotsford Rise, Livingston, West Lothian EH54 6QD, UK

† Department of Zoology, The University, Newcastle upon Tyne NE1 7RU, UK

Despite several important discoveries, extending over more than 120 years, our knowledge of early land vertebrates is still sparse. The earliest tetrapod remains are known from the Upper Devonian of East Greenland¹⁻³ and Australia⁴⁻⁶, but the tetrapod fossil record does not become plentiful until Coal Measure times, in the Upper Carboniferous, some 50 Myr later. Finds in the Lower Carboniferous are very few indeed. Apart from two localities in West Virginia, USA^{7,8}, and one in Nova Scotia, Canada⁹, all other Lower Carboniferous tetrapod sites are from the Viséan of Fife and the Lothian Region, Scotland^{10,11}. We report here the discovery of an assemblage of terrestrial animals from a new Lower Carboniferous locality in the Lothian Region. Specimens were collected from the East Kirkton Limestone in the Brigantian stage of the Scottish Viséan, and include the first articulated amphibian skeleton to be found in the Lower Carboniferous of Europe in the twentieth century. This find is the earliest well-preserved amphibian skeleton ever discovered. The associated fauna is remarkable for the presence of myriapods, scorpions, the earliest known harvestman and several other types of amphibian. The presence of such forms, together with the striking absence of fishes, suggests that the amphibians form an integral part of a terrestrial fauna; terrestrial amphibians are otherwise unknown before the Upper Carboniferous Coal Measures.

The earliest Lower Carboniferous tetrapod sites are in the Oil Shale Group (Fig. 1), and the fauna consists of the aïstopod *Lethiscus*¹², the colosteoid temnospondyl *Pholidogaster*¹³ and a form of unknown affinities, *Doragnathus*¹⁴. There is also a series of specimens, including three incomplete skeletons, which are attributable to the Adelogyrinidae¹⁵. The principal amphibian find reported here is not attributable to any of these taxa. The site from which it comes is a new one in the Lothian Region. The specimen was found in the East Kirkton Limestone in sediments originating from the East Kirkton Quarry, near Bathgate, West Lothian. The limestone is very localized and separated by the Riccarton Hills Lavas from the overlying Hurlet Limestone, which marks the boundary between the Upper Oil Shale Group and the Lower Limestone Group (Fig. 1)¹⁶. The underlying Bankhead Tuffs separate it from the Raeburn Shell Bed, which in West Lothian probably forms the base of the Brigantian¹⁷.

In striking contrast to other Scottish Mississippian tetrapod localities, there is a complete absence of fish remains at this horizon. The matrix in which the amphibians were found is a peculiar laminated cherty limestone¹⁸, and initial associated discoveries suggest a unique environment of deposition. Most notable among these is what appears to be the earliest known fossil harvestman (Arachnida: Opiliones)¹⁹ (Fig. 2). A number of myriapods and scorpions have also been found. Together with these small arthropods are several incomplete specimens

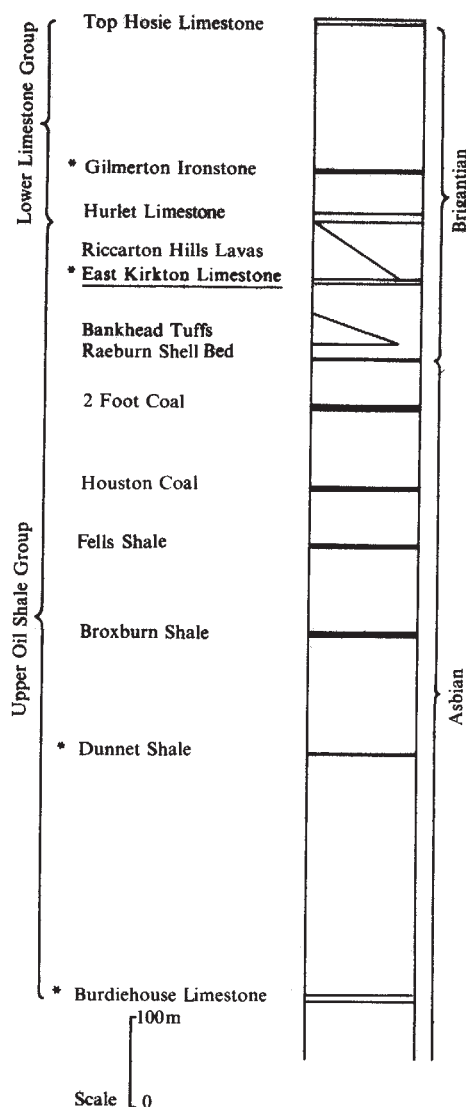


Fig. 1 Generalized section of the Upper Viséan of West Lothian, Scotland (after Geological Survey of Great Britain (Scotland), Sheet 32W). *Amphibian horizons.

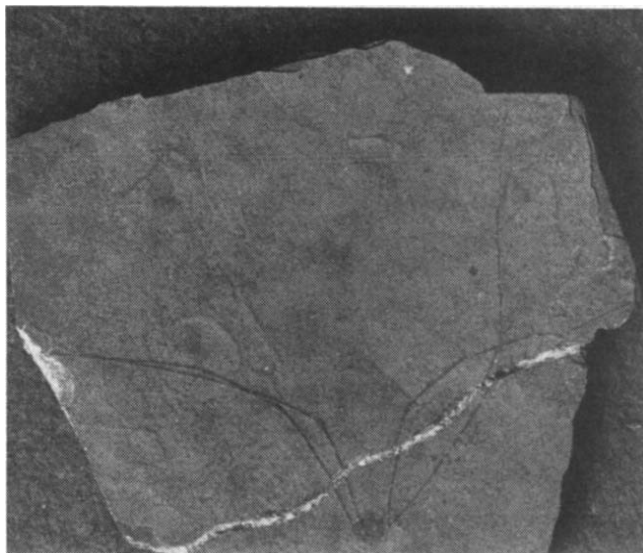


Fig. 2 Fossil harvestman (Opiliones) from the East Kirkton Limestone (SPW: G480). Natural size.

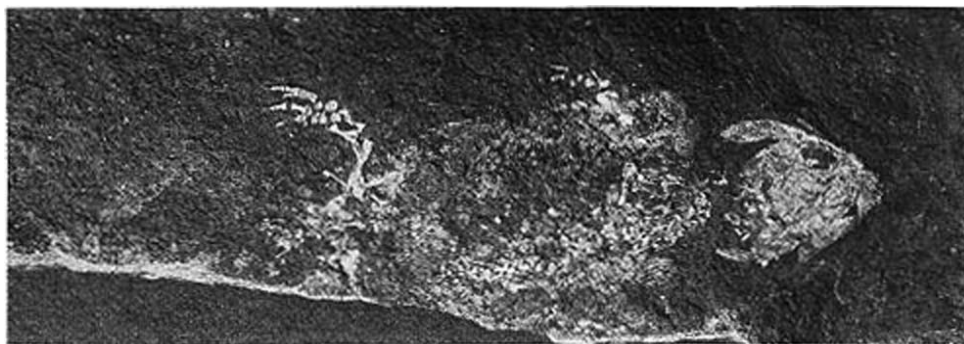


Fig. 3 The earliest complete fossil amphibian skeleton (SPW: 2042A). Dorsal view ($\times \sim 0.4$).

of gigantic eurypterids. *Hibbertopterus scouleri* has already been described from the East Kirkton Limestone²⁰, but the new material cannot be referred to any Scottish Lower Carboniferous species.

The principal find (field no. SPW: 2042 A/B) consists of a tetrapod skeleton of some 40 cm overall length. It is preserved in part and counterpart on two blocks of limestone. The principal block (Fig. 3) shows the skeleton as it was when originally collected. The skull, vertebral column, girdles and limbs are all in natural articulation and the specimen is completed by the tail, which is defined by its investing scales. The specimen is remarkable for the intact preservation of the hands and feet, with ossified carpals and tarsals, and should greatly augment our understanding of the early evolution of the tetrapod limb.

The skull is similar to those of edopoid and dissorophoid temnospondyls from the Upper Carboniferous and Lower Permian^{21,22}. The orbits and interpterygoid vacuities are relatively large, the otic notches are distinct and there is no visible trace of a lateral line canal system. However, the sutures between the bones of the skull roof and palate are difficult to trace and a detailed study will be necessary before the relationships of this new species can be established. The vertebrae appear to be of

the primitive temnospondyl type, with separate intercentra and pleurocentra surrounding a persistent notochord. The general morphology of the appendicular skeleton is also similar to that of temnospondyls, with a four-digit manus and five-digit pes, but the structure of the carpus and tarsus, as well as the phalangeal formulae of the hands and feet, remain to be determined. Six other specimens from the East Kirkton Limestone are provisionally attributed to this species. They include a skull of similar size to that of the complete skeleton (Fig. 4), a pelvis with paired hind limbs, and two incomplete skeletons.

A second temnospondyl species is represented in the fauna by an incomplete and poorly preserved skeleton. It differs from the complete skeleton illustrated in Fig. 3 in being much shorter-bodied and with limbs of different proportions. Anthracosaur amphibians have also been found. Caudal vertebrae, similar to those of *Proterogyrinus*²³, have been collected, together with two incomplete humeri which resemble closely those of *Eoherpeton*²⁴.

There is no sign in the complete amphibian skeleton of an aquatic mode of life; lateral-line canals seem to be absent and the skeleton, particularly that of the limbs, is remarkably heavily ossified. This is also true of the other amphibian remains, and, in the light of the associated arthropods, it seems probable that they represent the only known pre-Coal Measure terrestrial vertebrate fauna. Complete preparation and description of this new material is under way, but will take some time. Meanwhile, we anticipate that further exploration of the East Kirkton Limestone will yield more evidence of the early Carboniferous land fauna. It is hoped that this will result in the discovery of the forerunners of the many amphibian groups which appear suddenly in the Upper Carboniferous. We do not exclude the possibility of the discovery of the earliest reptiles.

We thank Drs C. Burton and W. D. I. Rolfe (Glasgow University) for identifying the fossil arthropods and Dr A. R. Milner (Birkbeck College) for valuable criticism of the manuscript.

Received 10 September 1984; accepted 10 January 1985.

1. Säve-Söderbergh, G. *Meddr Grönland* **94**, No. 7, 1-107 (1932).
2. Jarvik, E. *Ark. Zool.* **41A**, No. 13, 1-8 (1948).
3. Jarvik, E. *Meddr Grönland* **149**, No. 6, 1-20 (1950).
4. Warren, J. W. & Wakefield, N. A. *Nature* **238**, 469-470 (1972).
5. Campbell, K. W. S. & Bell, M. W. *Alcheringa* **1**, 369-381 (1977).
6. Warren, A. A. *The Fossil Vertebrate Record of Australasia* (eds Rich, P. V. & Thompson, E. M.) 145-157 (Monash University Press, 1982).
7. Romer, A. S. *Proc. Am. phil. Soc.* **100**, 157-167 (1956).
8. Smithson, T. R. *Zool. J. Linn. Soc.* **76**, 29-90 (1982).
9. Carroll, R. L. *et al. Field Excursion A59: Guidebook* (24th int. Geol. Congr., Montreal, 1972).
10. Paton, R. L. R. *Scott. Mus. Inform. Ser. Geol.* **5**, 1-38 (1975).
11. Andrews, S. M., Browne, M. A. E., Panchen, A. L. & Wood, S. P. *Nature* **265**, 529-532 (1977).
12. Wellstead, C. F. *Palaeontology* **25**, 193-208 (1982).
13. Panchen, A. L. *Phil. Trans. R. Soc. B259*, 581-640 (1975).
14. Smithson, T. R. *Palaeontology* **23**, 915-923 (1980).
15. Brough, M. C. & Brough, J. *Phil. Trans. R. Soc. B252*, 107-165 (1967).
16. Mitchell, G. H. & Mykura, W. *The Geology of the Neighbourhood of Edinburgh* (HMSO, Edinburgh, 1962).
17. George, T. N. *et al. A Correlation of Dinantian Rocks in the British Isles* (Geological Society, London, 1976).
18. Muir, R. O. & Walton, E. K. *Trans. geol. Soc. Glasg.* **22**, 157-168 (1957).
19. Petrunkevitch, A. *Treatise on Invertebrate Paleontology* Pt P, Vol. 2 (ed. Moore, R. C.) 44-162 (Geological Society of America, Kansas, 1955).
20. Waterston, C. D. *Trans. R. Soc. Edinb.* **63**, 265-288 (1982).
21. Carroll, R. L. *J. Paleont.* **41**, 111-142 (1967).
22. Milner, A. R. *Palaeontology* **25**, 635-664 (1982).
23. Holmes, R. *Phil. Trans. R. Soc. B306*, 431-524 (1984).
24. Smithson, T. R. *Zool. J. Linn. Soc.* (in the press).

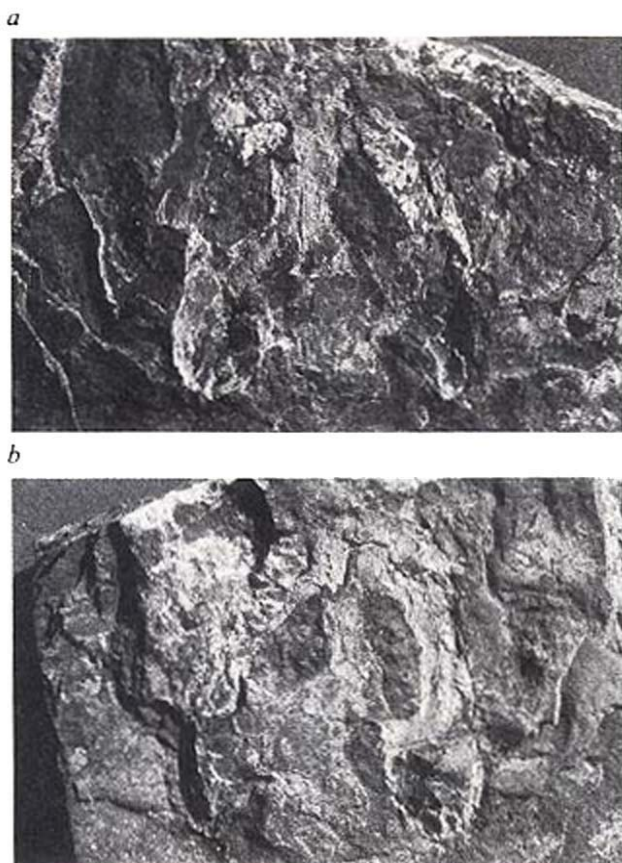


Fig. 4 Fossil amphibian skull (SPW: 2049). a, Skull roof, ventral view; b, palate, dorsal view. Natural size.

Multiple innervation of tonic endplates revealed by activity-dependent uptake of fluorescent probes

Jeff W. Lichtman, Robert S. Wilkinson & Mark M. Rich

Department of Physiology and Biophysics, Washington University School of Medicine, 4566 Scott Ave., St Louis, Missouri 63110, USA

During development of the vertebrate nervous system, there is a widespread reduction in the number of axons innervating target cells¹. This phenomenon, often called synapse elimination, has been particularly well studied at the neuromuscular junction of developing twitch muscle fibres^{2,3}: following a period of polynuclear innervation, axonal branches are retracted, usually leaving each twitch fibre endplate innervated by only one axon. Here we describe a new technique for the study of synapse elimination—activity-mediated uptake of fluorescent probes. These probes selectively and supravitaly label all the terminals of individual axons. The technique is used here in adult and embryonic snakes to study the innervation pattern of a thin muscle containing two fibre types: twitch fibres, which are fast-contracting and have propagated action potentials, and tonic fibres, which are slow-contracting and lack action potentials. We find that twitch muscle fibres, as expected, eliminate all polynuclear innervation during development; in contrast, tonic fibre endplates remain polynuclearly innervated into adulthood. The persistence of multiple innervation at tonic endplates may be related to the lack of action potential activity in tonic muscle fibres.

The labelling technique takes advantage of endocytosis at the synaptic terminals of stimulated axons⁴ to accumulate fluorescent molecules at synapses. Our method allows living terminals to be viewed with light microscopy, but, unlike other supravital techniques^{5,6}, the synapses of different axons in the same preparation can be stained selectively with different-coloured probes.

We have at present three fluorescent probes that are internalized selectively by active nerve terminals; they are sulphonate acid derivatives of fluorescent molecules of low relative molecular mass (rhodamine, fluorescein and 8-hydroxypyrene; see Fig. 1 legend). Each probe emits at a different peak wavelength (red, green and blue, respectively). Brief stimulation (5–10 min) of a muscle nerve in a bath containing one of the probes labels all the terminals of the stimulated motor axons. Sequential stimulation of individual motor axons in the presence of different-coloured probes allows the relationship among terminals of different axons to be visualized.

The innervation of twitch and tonic muscle fibre endplates was examined in the transversus abdominis muscle of the garter snake; this muscle comprises ~200 segmental components, each of which extends from a rib to the ventral midline of the animal⁷. Each segmental component is supplied by a nerve containing 4–5 twitch and 3–4 tonic motor axons. The muscle is only one fibre thick, thus all the nerve–muscle contacts are visible in living preparations. A useful feature of this muscle is that the segmental components form an uninterrupted sheet, so that adjacent segmental nerves occasionally innervate the same band of endplates. By stimulating one segmental nerve in the presence of one fluorescent probe and then stimulating the adjacent nerve in the presence of a different-coloured probe, we could label separately all the terminals of two populations of axons in the same endplate band in either adult or embryonic snakes.

Initially the probes were used to study the pattern of innervation at the border region between adjacent segmental muscles. The results (Fig. 1a) showed that twitch fibre endplates innervated by one segmental nerve (red) are interspersed with endplates innervated by the adjacent segmental nerve (green). None of the individual twitch fibre endplates showed a mixture of red and green boutons, which would have indicated innervation by axons from both nerves.

In contrast, tonic muscle fibre endplates in adult snakes showed a very different pattern of labelling: when one of the segmental nerves was stimulated in the presence of the red probe and the adjacent nerve in the presence of the green probe, many of the tonic fibre endplates near the segmental border contained a mixture of both red and green synaptic boutons (Fig. 1b). Such 'mixed' endplates were found in each of 15 preparations in which there was a continuous endplate band crossing segmental boundaries. This observation indicates that, in maturity, tonic fibre endplates near the segmental boundaries are frequently innervated by axons from both segments.

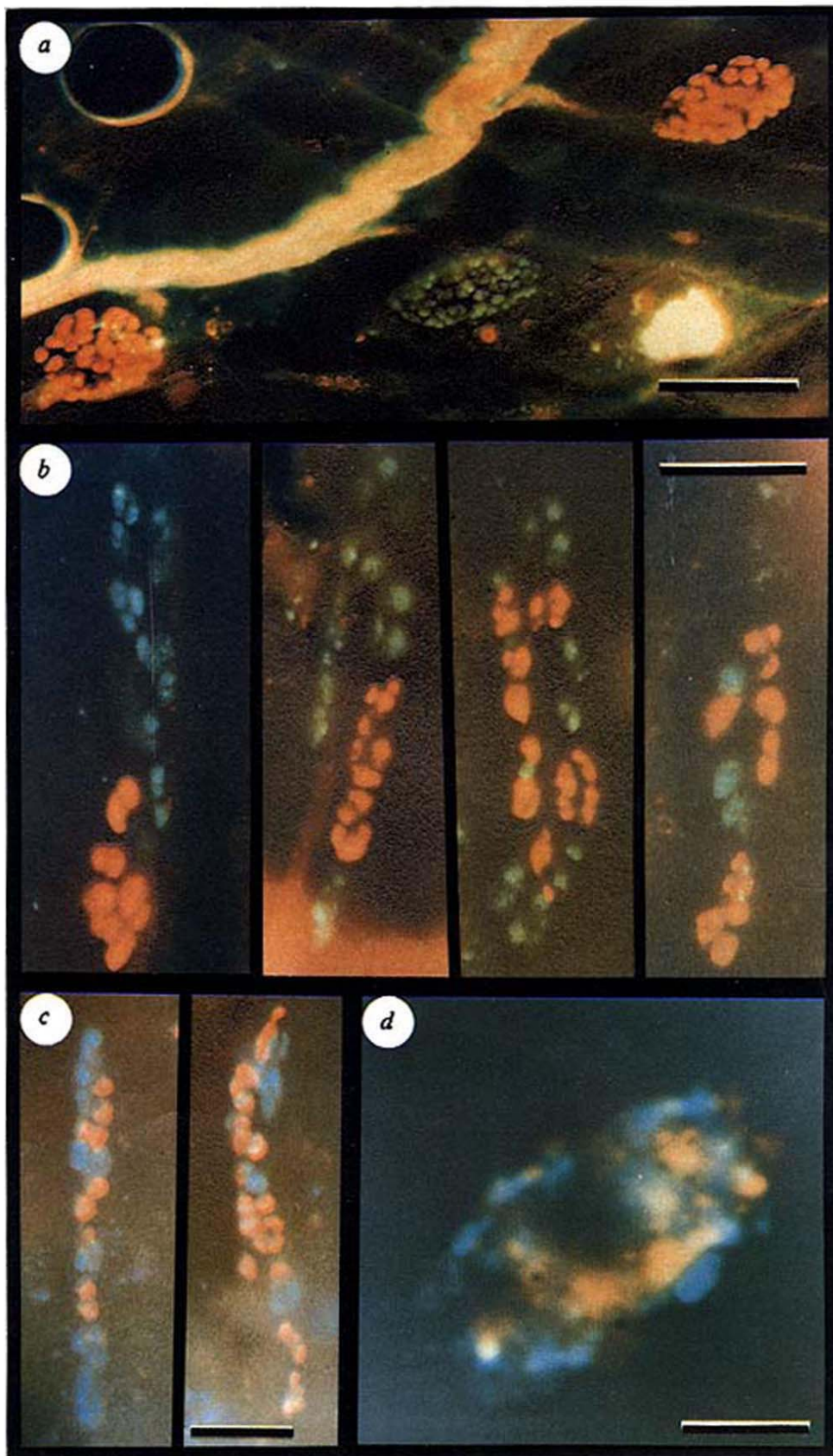
To determine whether multiple innervation is typical of tonic fibre endplates throughout the muscle rather than just at the borders, we labelled a single tonic motor unit with the red probe, and all the other motor units within that segment with the blue probe (Fig. 1c). In this way we could examine every endplate site contacted by a single tonic motor axon. Each of two tonic units (from two snakes) studied in this way contributed 25–30% of synaptic boutons on the tonic muscle fibres in the muscle (1,477/5,539 and 1,305/4,571), yet each innervated 44% of the total number of tonic fibre endplates (98/223 and 102/234), suggesting that many of the endplates are multiply innervated. In fact, in both motor units, most of the endplates having red boutons, and thus innervated by the separately stimulated motor unit (85% and 54%), were also innervated by other axons as demonstrated by the presence of blue boutons at the same endplate (Fig. 1c). In contrast, three experiments in which a twitch motor unit was selectively labelled in the same manner as the tonic units (see above) revealed uniformly single innervation of twitch fibres: none of the twitch fibre endplates having red boutons, and thus within the separately stimulated twitch motor unit, also had blue boutons.

To estimate the number of axons that converge at a tonic fibre endplate, the numbers of red and blue boutons were counted at each tonic endplate site. The fraction of the total number of boutons contributed by the separately stimulated motor unit [red/(red + blue)] indicates the percentage of that endplate supplied by one axon and thus provides an approximation of the number of innervating axons. At multiply innervated endplate sites (those with both red and blue boutons), the identified motor axon formed, on average, less than half of the boutons (46% and 23%), arguing that many tonic fibre endplates are doubly innervated and occasional endplates are innervated by more than two axons.

The anatomical evidence for single innervation of twitch fibres and multiple innervation of tonic fibres was confirmed electrophysiologically. Intracellular recordings at the endplate sites of 50 twitch fibres (paralysed with curare or formamide⁸) always showed a single step in the postsynaptic response to a gradually increasing strength of stimulus to the muscle's nerve; a single step indicates recruitment of only one innervating axon (Fig. 2a). Physiological confirmation of multiply innervated tonic fibre endplates is more complicated as each tonic muscle fibre has several endplates^{9,10}. Thus, both the presence of multiple steps in the postsynaptic response to gradually increasing nerve stimulation and the risetime of these steps must be considered to obtain unambiguous evidence for multiple inputs at a single endplate site. Intracellular recordings from tonic muscle fibres immediately adjacent to one endplate site (located with Nomarski optics) showed the presence of such multiple innervation (Fig. 2b–d). Of 57 tonic endplates studied electrophysiologically, 49% seemed to be singly innervated (see Fig. 2b), 44% doubly (see Fig. 2c) and 7% triply innervated (Fig. 2d), all consistent with the degree of multiple innervation assessed anatomically.

Studies of developing snake muscles suggest that the differing innervation patterns of twitch and tonic fibre endplates arise during development because of more vigorous synapse elimination from twitch than from tonic muscle fibres. The transversus abdominis muscle was dissected from late embryonic snakes (stages 35–36)¹¹. The activity-selective fluorescent probes worked well in embryos, although endplates were smaller than

Fig. 1 Photomicrographs of activity-mediated uptake of fluorescent probes by motor axon terminals in the adult and embryonic transversus abdominis muscle of the garter snake *Thamnophis sirtalis*. **a**, At the segmental boundary, adult twitch muscle fibres innervated by adjacent segmental nerves are interspersed. None of the twitch terminals studied was innervated by both segmental nerves. The figure shows two red-labelled nerve terminals supplied by the more rostral segmental nerve (left); between them is a green terminal from the more caudal segmental nerve (right). The rostral segmental nerve was stimulated for 5 min (75 Hz, 3 s on/2 s off) in a bath containing Ringer's solution and sulphorhodamine 101 (0.1 mg ml⁻¹; Molecular Probes Inc., Junction City, Oregon). The preparation was washed in Ringer's for 30 min, placed in Ringer's containing 0.1 mg ml⁻¹ fluorescein 5,6-sulphonic acid (Molecular Probes Inc.), and the adjacent (caudal) nerve stimulated in the same manner. The preparation was washed for 30 min, fixed in 4% paraformaldehyde for 30 min and mounted on a slide in *p*-phenylenediamine phosphate-buffered saline/glycerin solution²³ to prevent fading. Living material may also be used but exciting light levels must be kept low to prevent bleaching and damage. Calibration bar, 35 μ m. **b**, Individual tonic endplates at the border of adjacent segmental muscles are frequently innervated by axons from both segmental nerves. With the same stimulus paradigm as in **a**, most tonic endplates in the border region showed a mixture of green and red boutons. Farther from the segmental border, tonic endplates containing both types of boutons declined, becoming all red in the rostral segment and all green in the caudal segment. Calibration bar, 10 μ m. **c**, The synaptic contribution of one motor axon to multiply innervated tonic endplates. To assess the proportion of synapses contributed by one axon in a multiply innervated endplate, the boutons of all the motor axons were labelled with the blue fluorescent probe 8-hydroxypyrene trisulphonic acid (Eastman Organic Chemicals, Rochester, New York) by stimulating the segmental nerve at supramaximal strength (same procedure as above); boutons of a single tonic motor axon were subsequently filled with the red probe (and, concomitantly, partially depleted of the blue probe) by selectively restimulating it alone in the presence of sulphorhodamine 101. A single tonic motor unit was activated by locating a tonic axon near an endplate (with Nomarski microscopy) and stimulating it with an extracellular electrode. This stimulation technique retrogradely activates the entire motor unit²⁴; extracellular recording from the proximal nerve showed a single action potential, thus only one motor axon was active. The figure shows two of the 102 endplates innervated by the stimulated tonic axon (the boutons of the separately stimulated axon are red; the rest are blue). Calibration bar, 10 μ m. **d**, Multiple innervation of a twitch endplate in an embryonic snake muscle. Using the same stimulus procedure as in **a** and **b**, the motor axons from adjacent segments were labelled with the red and blue probes. Although all endplates were smaller than in adults, twitch endplates were still recognizable by virtue of their round shape and larger size than tonic endplates. Unlike adult preparations, embryonic twitch endplates at the segmental border received input from both segments, indicating innervation by more than one axon. Calibration bar, 5 μ m. **a-c**, Photomicrograph double exposures using two sets of barrier and excitation filters. **d**, Photograph of a digitally stored video image.



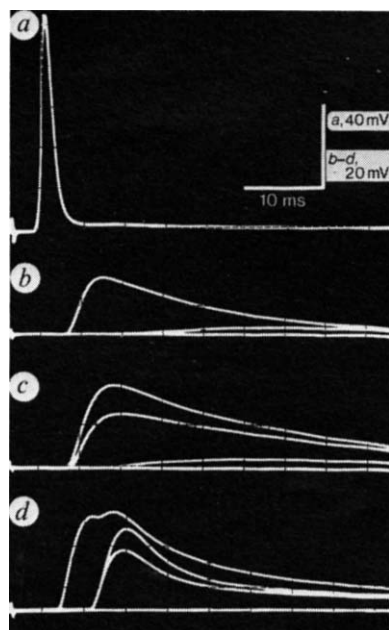


Fig. 2 Intracellular recordings from adult twitch and tonic muscle fibres in the transversus abdominis muscle of garter snakes, treated with formamide to induce muscle paralysis without reducing the amplitude of synaptic potentials⁸. *a*, Twitch fibres were identified by their large endplates. Recordings near the endplate always showed a single, large regenerative response in the postsynaptic potential. The unitary postsynaptic response is evidence of innervation by a single motor axon. Similar results were obtained with curare added to the bath in sufficient concentration to make synaptic potentials subthreshold. *b-d*, In tonic fibres, multiple steps in the postsynaptic response to nerve stimulation were often seen. Each panel is an intracellular record from a different tonic muscle fibre; all recordings were made adjacent to a tonic endplate viewed with Nomarski optics. The neighbouring endplate site was singly innervated in *b*, doubly innervated in *c*, and triply innervated in *d*. We interpret the large-amplitude, fast-rising steps as inputs to the nearby endplate, whereas the smaller and slower rising synaptic potentials are presumably inputs to the adjacent endplate sites (at a distance of ~1 mm). No potentials intermediate in risetime and amplitude to the two classes shown here were seen, suggesting that the source of the slow-rising potentials was an adjacent endplate and that more distant synaptic inputs were below the resolution level of our recordings. None of the tonic fibres showed regenerative membrane responses.

in adults and individual synaptic boutons were more difficult to discern. In contrast to the innervation of adult muscles (Fig. 1*a*), both twitch and tonic muscle fibre endplates at the segmental boundary in embryonic muscles showed innervation from both segmental nerves (Fig. 1*d*). Multiple innervation of twitch fibres was found in each of five embryonic muscles studied. However, by 1 week after birth (10 animals) no evidence of multiple innervation of twitch fibre endplates was found using either the fluorescent probes or intracellular recording. This result indicates that synapse elimination at twitch fibre endplates effectively removes all but one input to these muscle fibres perinatally. Tonic muscle fibre endplates, however, showed evidence of polyneuronal innervation throughout development and into adulthood.

It is uncertain why tonic endplates should retain multiple innervation. One possible explanation is suggested by an observation on doubly innervated¹² neuromuscular junctions of some frog twitch fibres—these multiply innervated sites are unusual because the inputs are weak and, when repetitively stimulated, are often unable to depolarize the muscle fibre sufficiently to generate action potentials¹². Thus, the persistence of multiple innervation at tonic fibre endplates may be related to the fact that tonic muscle fibres generally lack action potential activity¹⁰ regardless of the amplitude of their synaptic inputs

(see Fig. 2). Indeed, several experiments suggest that the activity of postsynaptic cells influences synapse elimination^{13–20}, perhaps by allowing the postsynaptic cell to reward some inputs trophically at the expense of others^{21,22}. The ability to view selectively the terminals of living axons undergoing synaptic rearrangement should provide a direct experimental approach to resolving this problem.

We thank Drs C. Forehand, D. Purves and J. Sanes for critical comments. This work was supported by grants from the NIH and Muscular Dystrophy Association. J.W.L. is a Sloan Fellow.

Received 5 November 1984; accepted 22 January 1985.

1. Purves, D. & Lichtman, J. W. *Science* **210**, 153–157 (1980).
2. Van Essen, D. C. in *Neuronal Development* (ed. Spitzer, N. C.) 333–376 (Plenum, New York, 1982).
3. Brown, M. C. et al. in *Development of the Nervous System* (eds Garrod, D. R. & Feldman, J. D.) 245–262 (Cambridge University Press, 1981).
4. Heuser, J. E. & Reese, T. S. *J. Cell Biol.* **57**, 315–344 (1973).
5. Yoshikami, D. & Okun, L. M. *Nature* **310**, 54–56 (1984).
6. Wilcox, M. & Franceschini, N. *Science* **225**, 851–854 (1984).
7. Wilkinson, R. S. & Lichtman, J. W. *J. Neurosci.* (submitted).
8. Córdoba, F. et al. *J. Physiol., Lond.* **291**, 73–74 p (1979).
9. Bennett, M. R. & Pettigrew, A. G. *J. Physiol., Lond.* **241**, 515–545 (1974).
10. Hess, A. *Physiol. Rev.* **50**, 40–62 (1970).
11. Zehr, D. R. *Copeia* **2**, 322–329 (1962).
12. Angaut-Petit, D. & Mallart, A. *J. Physiol., Lond.* **289**, 203–218 (1979).
13. Benoit, P. & Changeux, J.-P. *Brain Res.* **99**, 354–358 (1975).
14. Riley, D. A. *Brain Res.* **143**, 162–167 (1978).
15. O'Brien, R. A. D. et al. *J. Physiol., Lond.* **282**, 571–582 (1978).
16. Thompson, W. et al. *Neuroscience* **4**, 271–278 (1979).
17. Brown, M. C. et al. *J. Physiol., Lond.* **318**, 355–364 (1981).
18. Duxson, M. J. *J. Neurocytol.* **11**, 395–408 (1982).
19. Thompson, W. *Nature* **302**, 614–616 (1983).
20. Ridge, R. M. A. P. & Betz, W. J. *J. Neurosci.* **4**, 2614–2620 (1984).
21. Changeux, J.-P. & Danchin, A. *Nature* **264**, 705–712 (1976).
22. Purves, D. & Lichtman, J. W. *Principles of Neural Development* (Sinauer, Sunderland, Massachusetts, 1984).
23. Johnson, D. G. et al. *J. Immun. Meth.* **43**, 349–350 (1981).
24. Ridge, R. M. A. P. *J. Physiol., Lond.* **217**, 393–418 (1971).

Activation of protein kinase C potentiates isoprenaline-induced cyclic AMP accumulation in rat pinealocytes

David Sugden, Jiri Vanecek, David C. Klein, Thomas P. Thomas* & Wayne B. Anderson*

Section on Neuroendocrinology, Laboratory of Developmental Neurobiology, National Institute of Child Health and Human Development and * Laboratory of Tumor Immunology and Biology, National Cancer Institute, Bethesda, Maryland 20205, USA

The pineal gland has proven to be an excellent model for the study of adrenergic control systems¹. Noradrenaline, released from sympathetic nerve terminals in the pineal gland, regulates a large nocturnal increase in melatonin synthesis by stimulating the activity of arylalkylamine *N*-acetyltransferase (NAT, EC 2.3.1.87) 30–70-fold^{1,2}. An essential step in both the induction and maintenance of high NAT activity is an increase in intracellular cyclic AMP^{3,4}. Noradrenaline acts via β -adrenoceptors to increase pineal cyclic AMP by activating adenylate cyclase⁵, and the activation of pineal α_1 -adrenoceptors⁶ potentiates β -adrenergic stimulation not only of NAT^{7,8} but of both cyclic AMP and cyclic GMP^{9–11}. Here we describe investigations designed to test whether α_1 -adrenergic potentiation of β -adrenergic stimulation of pineal cyclic AMP involves protein kinase C. Our results suggest that kinase activation is involved and the data provide the first demonstration of a synergistic interaction between Ca^{2+} -phospholipid-dependent protein kinase (protein kinase C) and neurotransmitter-dependent stimulation of cyclic AMP.

Pinealocytes were prepared from female Sprague–Dawley rats (5 weeks old, 120 g) by trypsinization¹² and incubated (10^5 cells per 0.5 ml, 37 °C, 95% O_2 /5% CO_2) in suspension culture in Dulbecco's modified Eagle's medium (DMEM) containing fetal calf serum (10% v/v) for 5–7 h before use. Following the indicated treatments, cells were centrifuged (8,000g, 1 min), the

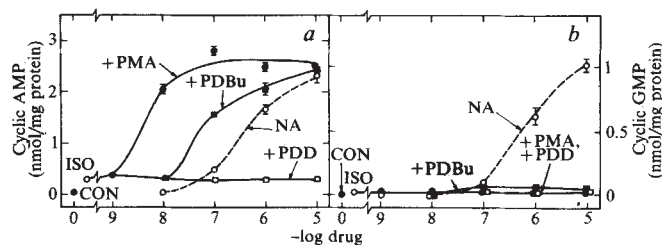


Fig. 1 Potentiation of β -adrenergic cyclic nucleotide responses by phorbol esters (a, cyclic AMP; b, cyclic GMP). Pinealocytes (10^5 cells per tube) were treated for 15 min with (—)isoprenaline (ISO; 10^{-6} M) either alone or in combination with 4- β -phorbol 12-myristate 13-acetate (PMA), 4- β -phorbol 12,13-dibutyrate (PDBu) or 4- α -phorbol 12,13-didecanoate (PDD). The dose-response curve to (—)noradrenaline (NA) is shown for comparison. Phorbol esters (Sigma) were added as $100\times$ concentrated solutions in ethanol. All points represent the mean $\pm$ s.e.m. of three samples. The lack of error bars indicates that the s.e.m. was less than the area covered by the symbol.

media aspirated and the cell pellet immediately frozen on dry ice. Samples were sonicated in perchloric acid (5%), neutralized, then frozen (-30°C) until cyclic nucleotide concentrations were determined in duplicate by radioimmunoassay following acetylation¹³. The cross-reactivities of the cyclic AMP and cyclic GMP antisera were 0.07% and 0.53% respectively. Protein was determined by a dye binding assay using bovine serum albumin as the standard¹⁴.

Treatment with isoprenaline (10^{-6} M), a β -adrenergic agonist, produced an eightfold increase in pinealocyte cyclic AMP and a doubling of cyclic GMP. Addition of 4- β -phorbol 12-myristate 13-acetate (PMA) or 4- β -phorbol 12,13-dibutyrate (PDBu), activators of protein kinase C¹⁵, together with isoprenaline, potentiated the cyclic AMP, but not the cyclic GMP, response (Fig. 1). The maximal increase in cyclic AMP was comparable to that seen with noradrenaline, a mixed α - and β -adrenergic agonist. The concentrations of PMA and PDBu producing half-maximal potentiation were approximately 5 and 50 nM respectively. These concentrations are similar to the reported half-maximal concentrations necessary to activate protein kinase C¹⁶. 4- α -Phorbol 12,13-didecanoate (PDD), which does not activate protein kinase C, had no potentiating effect (Fig. 1). Addition of these phorbol esters alone produced no elevation in cyclic AMP.

An examination of the time course of the cyclic AMP response to isoprenaline (10^{-6} M) or isoprenaline plus PMA (10^{-7} M) indicated that potentiation was present after 1 min, reached a peak by 10 min and was still elevated after 1 h (Fig. 2). The small potentiation of the cyclic GMP response is probably an artefact caused by the cross-reactivity of the cyclic GMP antisera.

Measurement of protein kinase C activity in pinealocytes revealed a very high activity (42,000 pmol ^{32}P incorporated per 3 min per mg protein) when compared with most tissue^{17,18} including neural tissues (Table 1). Treatment of cells with either phenylephrine (10^{-6} M) or PMA (10^{-7} M) caused a rapid decrease in cytosolic protein kinase C activity with a corresponding increase in membrane-associated activity. It has been suggested that the redistribution of this protein kinase activity may be an important, early event in the action of phorbol esters, perhaps enabling the enzyme to gain access to physiological substrates¹⁹.

The physiological activator of protein kinase C is thought to be diacylglycerol, which is transiently formed from the breakdown of membrane phosphatidylinositol²⁰. Noradrenaline, acting through α_1 -adrenoceptors, is known to stimulate this process in the pineal gland^{21,22}. A synthetic diacylglycerol analogue, 1-oleoyl 2-acetyl glycerol (OAG), can also activate C kinase²³ and furthermore is able to cross the cell membrane. OAG (12.5 – $50\ \mu\text{g ml}^{-1}$) potentiated the stimulation of cyclic AMP produced by isoprenaline (10^{-6} M) (Fig. 3). No effect of OAG alone was observed. A small but significant potentiation of the cyclic GMP

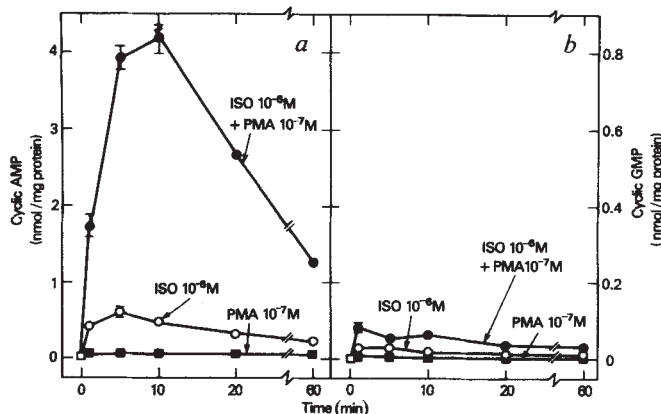


Fig. 2 Time course of PMA potentiation of β -adrenergic cyclic nucleotide response (a, cyclic AMP; b, cyclic GMP). Pinealocytes (10^5 cells per tube) were treated with PMA (10^{-7} M), (—)isoprenaline (10^{-6} M), or PMA (10^{-7} M) plus (—)isoprenaline (10^{-6} M) for the period indicated. All points represent the mean $\pm$ s.e.m. of three samples.

response to isoprenaline cannot be completely explained by the cross-reactivity of the cyclic GMP antisera.

These studies clearly show that phorbol esters and OAG, both of which activate protein kinase C, mimic the action of α_1 -agonists on pinealocytes and potentiate β -adrenergic stimulation of cyclic AMP content. In addition, protein kinase C is rapidly redistributed from the cytosol into membranes not only in response to PMA¹⁹, but also by treatment of the intact cells with an α_1 -agonist, phenylephrine. These findings, together with earlier evidence that postsynaptic α_1 -adrenoceptors mediate a rapid increase in membrane phosphatidylinositol turnover in pinealocytes^{21,22}, suggest that activation of protein kinase C is involved in the α_1 -adrenergic amplification of β -adrenergic stimulation of cyclic AMP accumulation in this cell. Presumably, diacylglycerol generated by noradrenaline-mediated phosphatidylinositol turnover interacts with protein kinase C in the

Table 1 Effect of phenylephrine or PMA treatment of pinealocytes on cytosolic and membrane-bound protein kinase C activity

Expt no.	Pretreatment	Protein kinase C activity (pmol ^{32}P incorporated per 3 min per 5×10^6 cells)	
		Cytosol	Membranes
1	Water	13,750	1,000
	Phenylephrine (10^{-6} M)	8,725	5,160
2	Vehicle	13,248	1,395
	PMA (10^{-7} M)	6,286	3,164

Pinealocytes (5×10^6) were treated with phenylephrine (10^{-6} M) for 1 min or with PMA (10^{-7} M) for 15 min. Cells were pelleted (1,000g, 1 min), washed, then lysed in water (250 μl) containing CaCl_2 (10^{-5} M) by brief (5-s) sonication (expt 1) or by repeated aspiration into a syringe through a fine (27-gauge) needle (expt 2). Membrane and cytosol were separated (12,000g, 1 min at 4°C) and 0.5 ml of buffer A (20 mM Tris-HCl, pH 7.5, 2 mM EDTA, 0.5 mM EGTA, 2 mM phenylmethylsulphonyl fluoride) was added to each preparation. Cytosol was applied to a DE-52 cellulose column (0.5 $\times$ 1 cm) equilibrated with buffer A. The column was washed with 8 volumes of buffer A, and the enzyme eluted with 0.08 M NaCl in buffer A. Membranes were solubilized with Nonidet P-40 (1%) by gentle mixing for 30 min at 4°C . The preparation was centrifuged (Beckman Microfuge, 10,000g, 2 min at 4°C) and the detergent-solubilized preparation applied to a DE-52 cellulose column and eluted as described for cytosol. Protein kinase C activity was assayed in triplicate as described previously with 0.75 mM Ca^{2+} , in the presence and absence of 24 μg phosphatidylserine, and 1.6 μg dioleoin^{17,30}. Protein kinase C activity was determined by subtracting the amount of ^{32}P incorporated into histone H1, in the absence of added phospholipids, from the amount incorporated in the presence of phospholipids. Protein kinase C activity is expressed as pmol ^{32}P incorporated per 3 min per total cytosolic and membrane fraction from 5×10^6 cells.

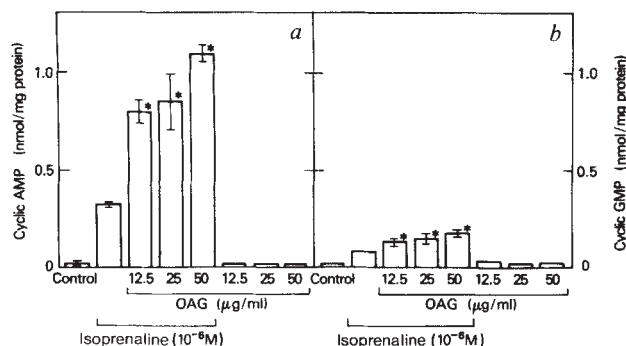


Fig. 3 Potentiation of β -adrenergic cyclic nucleotide responses by 1-oleoyl, 2-acetyl glycerol (OAG). OAG (10 mg ml⁻¹ in chloroform) was dried under N₂, then suspended in dimethyl sulphoxide (1%) by brief, vigorous sonication on ice immediately before use. OAG (a gift from Dr Y. Nishizuka) was added as $\times 25$ to $\times 100$ concentrated stock suspension to give the final concentrations indicated. Pinealocytes (10⁵ cells per tube) were pelleted (1,000g, 1 min) and resuspended in DMEM without fetal calf serum, then treated for 5 min with OAG alone or in combination with (-)isoprenaline. Each bar represents the mean $\pm$ s.e.m. of three samples. * Significantly different from isoprenaline alone, $P < 0.05$ using Duncan's multiple range test.

presence of membrane phospholipids and Ca²⁺ transiently to activate and stabilize the enzyme in association with the membrane. This activation of protein kinase C may result in the rapid phosphorylation of a component of the system synthesizing (β -adrenoceptor, guanine nucleotide binding proteins, adenylate cyclase catalytic subunit ?) or degrading (phosphodiesterase ?) cyclic AMP, an important intracellular messenger.

Apparently a different mechanism mediates the α_1 -adrenergic amplification of the β -adrenergic cyclic GMP response. Unlike cyclic AMP, the accumulation of pineal cyclic GMP in response to noradrenaline is Ca²⁺-dependent²⁴. Conceivably the α_1 -adrenergic amplification of the cyclic GMP response requires not only protein kinase C activation but also an elevation of intracellular Ca²⁺. This may explain the significant potentiation of the cyclic GMP response by OAG; OAG would be expected to be rapidly metabolized to the corresponding phosphatidic acid²³, which may then increase intracellular Ca²⁺. Inositol trisphosphate²⁵ or phosphatidic acid²⁶ generated as a result of an increased phosphatidylinositol turnover may be physiological mediators of this action of α_1 -adrenoceptor stimulation. Alternatively, guanylate cyclase may be stimulated by arachidonic acid or a metabolite²⁷.

Finally, these studies are of special interest because, to our knowledge, they are the first to describe a synergistic interaction between these intracellular signalling systems. Previous work has generally suggested that activation of protein kinase C may reduce neurotransmitter or hormone-dependent cyclic AMP signals (for example, see refs 28, 29). Clearly, this is not the case in all cell types.

Received 9 October 1984; accepted 2 January 1985.

- Klein, D. C., Auerbach, D. A., Nambodiri, M. A. A. & Wheler, G. H. T. in *The Pineal Gland: Anatomy and Biochemistry* (ed. Reiter, R. J.) 199–227 (CRC Press, Boca Raton, Florida, 1981).
- Klein, D. C. & Weller, J. L. *Science* **169**, 1093–1095 (1970).
- Deguchi, T. *Molec. Pharmacol.* **9**, 184–190 (1973).
- Klein, D. C., Buda, M. J., Kapoor, C. L. & Krishna, G. *Science* **199**, 309–311 (1978).
- Zatz, M., Kebabian, J. W., Romero, J. A., Lefkowitz, R. J. & Axelrod, J. *J. Pharmac. exp. Ther.* **196**, 714–722 (1976).
- Sugden, D. & Klein, D. C. *Endocrinology* **114**, 435–440 (1984).
- Klein, D. C., Sugden, D. & Weller, J. L. *Proc. natn. Acad. Sci. U.S.A.* **80**, 599–603 (1983).
- Sugden, D., Weller, J. L., Klein, D. C., Kirk, K. L. & Creveling, C. R. *Biochem. Pharmacol.* **33**, 3947–3950 (1984).
- Auerbach, D. A., Klein, D. C., Woodard, C. & Auerbach, G. D. *Endocrinology* **108**, 559–567 (1981).
- Klein, D. C., Auerbach, D. A. & Weller, J. L. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4625–4629 (1981).
- Vanecek, J., Sugden, D., Weller, J. L. & Klein, D. C. *Endocrinology* (in the press).
- Buda, M. & Klein, D. C. *Endocrinology* **103**, 1483–1493 (1978).
- Harper, J. F. & Brooker, G. *J. Cyclic Nucleotide Res.* **1**, 207–218 (1975).
- Bradford, M. M. *Analyt. Biochem.* **72**, 248–254 (1976).
- Castagna, M. et al. *J. biol. Chem.* **257**, 7847–7851 (1982).

- Kraft, A. S., Anderson, W. B., Cooper, H. L. & Sando, J. J. *J. biol. Chem.* **257**, 13193–13196 (1982).
- Kuo, J. F. et al. *Proc. natn. Acad. Sci. U.S.A.* **77**, 7039–7043 (1980).
- Minakuchi, R., Takai, Y., Yu, B. & Nishizuka, Y. *J. Biochem., Tokyo* **89**, 1651–1654 (1981).
- Kraft, A. S. & Anderson, W. B. *Nature* **301**, 621–623 (1983).
- Kishimoto, A., Takai, Y., Mori, T., Kikkawa, U. & Nishizuka, Y. *J. biol. Chem.* **255**, 2273–2276 (1980).
- Berg, G. R. & Klein, D. C. *J. Neurochem.* **19**, 2519–2532 (1972).
- Smith, T. L., Eichberg, J. & Hauser, G. *Life Sci.* **24**, 2179–2184 (1979).
- Kaibuchi, K., Sano, K., Hoshijima, M., Takai, Y. & Nishizuka, Y. *Cell Calcium* **3**, 323–335 (1982).
- O'Dea, R. F. & Zatz, M. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3398–3402 (1976).
- Joseph, S. K., Thomas, A. P., Williams, R. J., Irvine, R. F. & Williamson, J. R. *J. biol. Chem.* **259**, 3077–3081 (1984).
- Ohsako, S. & Deguchi, T. *J. biol. Chem.* **256**, 10945–10948 (1981).
- Snider, R. M., McKinney, M., Forray, C. & Richelson, E. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3905–3909 (1984).
- Nishizuka, Y. *Nature* **308**, 693–698 (1984).
- Anderson, W. B., Estival, A., Tapiovaara, H. & Gopalakrishna, R. *Adv. Cyclic Nucleotide Res.* (in the press).
- Kraft, A. S. & Anderson, W. B. *J. biol. Chem.* **258**, 9178–9183 (1983).

Human granulocyte-macrophage colony-stimulating factor is a neutrophil activator

Richard H. Weisbart*, David W. Golde†, Steven C. Clark‡, Gordon G. Wong‡ & Judith C. Gasson†

* Department of Medicine, Veterans Administration Medical Center, Sepulveda, California 91343, USA

† UCLA School of Medicine, Los Angeles, California 90024, USA

‡ The Genetics Institute, Inc., Boston, Massachusetts 02115, USA

The polymorphonuclear leukocyte (PMN), or neutrophil, is the major host defence cell protecting the body against invasion by bacteria and fungi. Products of oxidative metabolism mediate PMN microbicidal and tumoricidal activity¹ but the mechanisms by which these pathways become activated are not well understood. We have previously described a human granulocyte-macrophage colony-stimulating factor (GM-CSF) of relative molecular mass (M_r) 22,000 that also inhibits neutrophil motility (NIF-T activity)². Because of its direct action on granulocytes, this lymphokine is a candidate for a neutrophil-activating factor. We have studied the effect of GM-CSF/NIF-T on superoxide anion generation in response to the bacterial chemo-attractant *N*-formylmethionyl-leucylphenylalanine (f-MLP)³, and report here that PMNs preincubated with either purified natural GM-CSF or biosynthetic (recombinant) GM-CSF showed increased (as much as fourfold) superoxide anion production in response to f-MLP. These results indicate that human GM-CSF is a neutrophil-activating factor.

A neutrophil migration-inhibition factor from T lymphocytes (NIF-T) produced by a human T-lymphoblast cell line (Mo) was characterized as an acidic glycoprotein of M_r 22,000 (22K) with specificity for binding PMN plasma membranes^{2,4–6}. Purified NIF-T was shown to have colony-stimulating activity, and expression of a cDNA for human GM-CSF demonstrated that the gene product has NIF-T activity², confirming the identity of these two proteins.

In the experiments reported here, we examined the effect of GM-CSF on superoxide anion production by neutrophils, measured as superoxide dismutase-inhibitable cytochrome *c* reduction⁷. PMNs incubated with Mo-conditioned medium alone for 2 h did not produce measurable superoxide anion. In 10 separate experiments, a mean ($\pm$ s.e.m.) of 2.1 ± 0.2 nmol superoxide anion per min per 10^6 PMNs was produced over 4 min in the presence of 10^{-8} M f-MLP. However, when PMNs were preincubated for 2 h with Mo-conditioned medium before addition of f-MLP, superoxide anion production was increased to a mean of 7.0 ± 0.4 nmol per min per 10^6 PMNs measured over 4 min. The results of a representative experiment are shown in Fig. 1.

The degree of enhancement of f-MLP-induced oxidative metabolism was dependent on the length of time for which the PMNs

were incubated with Mo-conditioned medium (Fig. 2). A maximum (fourfold) increase in cytochrome *c* reduction (measured over 5 min) occurred when PMNs were incubated with Mo-conditioned medium for 2 h before addition of f-MLP (Fig. 2).

GM-CSF was isolated sequentially from Mo-conditioned medium by lectin affinity chromatography, gel filtration chromatography and reverse-phase HPLC, with co-purification of GM-CSF² and neutrophil-activating factor (NAF) activities (data not shown). GM-CSF isolated by HPLC was subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Analysis of eluates from gel slices showed CSF and NAF activities corresponding to a 22K protein (Fig. 3). The NH₂-terminal amino-acid sequence of this protein is identical to that predicted by the nucleotide sequence of the human GM-CSF cDNA clone².

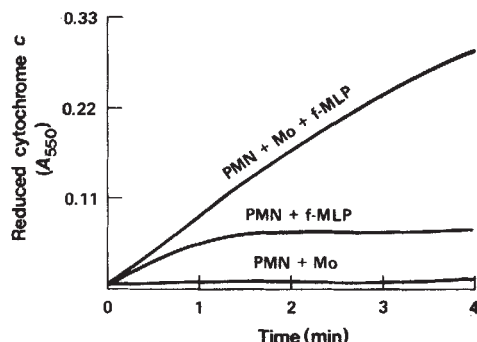


Fig. 1 Effect of Mo-conditioned medium on f-MLP-induced PMN oxidative metabolism.

Methods. PMNs were isolated from peripheral blood of healthy subjects by Ficoll-Hypaque gradient centrifugation, and red blood cells were removed by 6% dextran sedimentation and 30-s hypotonic lysis. PMNs ($5 \times 10^6 \text{ ml}^{-1}$) were suspended in Hanks' buffered salt solution (HBSS) (without phenol red) containing 5% fetal calf serum (FCS), and 0.4-ml aliquots containing 2×10^6 PMNs were incubated for 2 h at 37 °C with 0.040 ml of medium with 5% FCS alone or Mo-conditioned medium. After incubation, 2 ml of cytochrome *c* (1.2 mg ml^{-1} in HBSS with 5% FCS) were added to each cell suspension, mixed, and the contents divided equally into two tubes. Superoxide dismutase (0.010 ml of 2 mg ml^{-1}) was added to one of the tubes, and f-MLP was added as 1% of the volume to both tubes at a final concentration of 10^{-8} M . The cell suspensions were transferred immediately to disposable 1-ml cuvettes, and superoxide dismutase-inhibitable cytochrome *c* reduction was measured at 550 nm at 37 °C for 5 min in a Varian DMS 90 dual beam spectrophotometer. Absorbance was recorded every 5 s with a computer-assisted program. The data are expressed as the difference between results for Mo-conditioned medium and for medium alone.

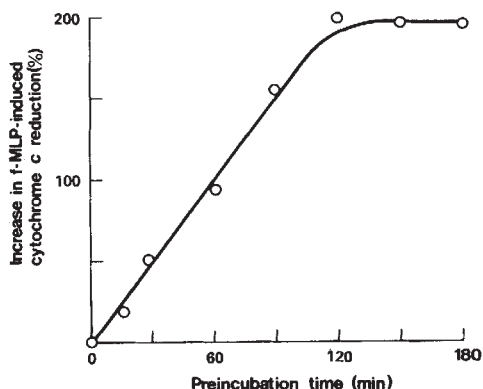


Fig. 2 Effect of length of preincubation with Mo-conditioned medium on f-MLP-induced PMN oxidative metabolism. Methods were the same as for Fig. 1 except that PMNs were preincubated for various periods of time with Mo-conditioned medium that titred 1:200 after 2 h of incubation. The data are expressed as percentage increase in mean A_{550} over 5 min after addition of f-MLP.

GM-CSF has been expressed in COS monkey cells transfected with a molecular clone of the human gene obtained independently from a cDNA expression library prepared from Mo mRNA^{2,8}. Such biosynthetic recombinant GM-CSF did not directly increase PMN oxidative metabolism. In 10 separate experiments, PMNs incubated with conditioned medium from mock-transfected COS cells produced a mean ($\pm$ s.e.m.) of $2.5 \pm 0.3 \text{ nmol}$ superoxide anion per min per 10^6 PMNs, measured over 4 min, in response to f-MLP. However, an average of $8.4 \pm 0.7 \text{ nmol}$ superoxide anion per min per 10^6 PMNs was produced over 4 min in response to f-MLP when PMNs were

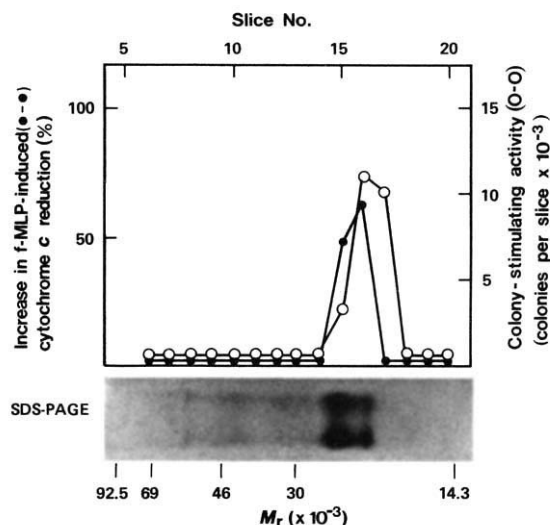


Fig. 3 Analysis of HPLC-purified CSF and NAF by SDS-PAGE. Ten litres of serum-free Mo-conditioned medium were concentrated and processed as described previously². Briefly, the proteins precipitated with 80% ammonium sulphate were dialysed and applied to a lentil lectin-Sepharose 4B column, washed, and eluted with 0.5 M α -methyl-D-mannoside. The eluted glycoproteins were fractionated on an Ultrogel Aca 44 column, and fractions with peak GM-CSF activity were pooled and further fractionated by reverse-phase HPLC using a Vydac C-18 column. Protein was eluted with a gradient of acetonitrile containing 0.1% trifluoroacetic acid. HPLC fractions were evaporated to dryness, reconstituted and assayed for NIF-T, CSF and NAF activities. A fraction previously shown to contain GM-CSF was assayed and found to have NAF activity; this fraction was subjected to SDS-PAGE in a 10% polyacrylamide gel². An aliquot of the sample was iodinated, fractionated with the unlabelled protein as a tracer, then analysed in parallel lanes in the presence and absence of 2-mercaptoethanol. The lane containing the unlabelled protein was sliced, eluted and assayed for NAF activity as well as NIF-T and CSF^{2,10,11}. The results obtained for NIF-T and CSF have been published previously².

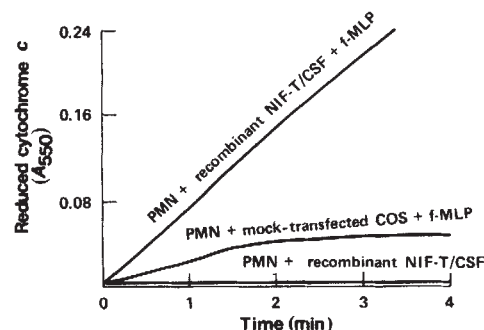


Fig. 4 Effect of biosynthetic recombinant GM-CSF on f-MLP-induced PMN oxidative metabolism. Computer-generated graph of measurements made at 5-s intervals. Methods as for Fig. 1, except that PMNs were preincubated with conditioned medium from monkey COS cells containing GM-CSF cloned⁸ in the expression vector p91023(B) and with medium from mock-transfected COS cells.

preincubated with recombinant GM-CSF. (Figure 4 shows the results of a representative experiment.) Furthermore, GM-CSF purified to homogeneity from COS-conditioned medium by gel filtration chromatography and HPLC had neutrophil-activating activity. Control experiments using buffers, medium and conditioned medium from mock-transfected COS cells, and other HPLC fractions, eliminated the possibility that endotoxin contamination was responsible for these results. Also, the titres of NIF-T, CSF and NAF activities were the same in Mo-conditioned medium (1:200) and conditioned medium from COS cells transfected with the cloned GM-CSF gene (1:10,000).

These studies demonstrate that GM-CSF enhances oxidative metabolism of PMNs in response to the chemo-attractant

f-MLP. Our results with this human lymphokine appear to correspond to those described for a murine CSF which has been shown to enhance granulocyte antibody-dependent cytotoxicity⁹. T-cell regulation of neutrophil oxidative metabolism by lymphokines may have an important role in neutrophil-mediated microbicidal and tumoricidal activity as well as in the pathogenesis of immune-mediated inflammatory diseases.

We thank Grace Chan, Amelia Kacena, Sue Kaufman, Shirley Quan and Noelle Bersch for technical assistance. This work was supported by USPHS grants CA30280 (R.H.W.), CA32737 (D.W.G., J.C.G. and R.H.W.), CA30388 (D.W.G. and J.C.G.) awarded by the NCI, DHHS, the Veterans Administration, and the Southern California chapter of the Arthritis Foundation.

Received 18 October 1984; accepted 23 January 1985.

1. Babior, B. M. *New Engl. J. Med.* **298**, 659-668, 721-725 (1978).
2. Gasson, J. C. *et al. Science* **226**, 1339-1342 (1984).
3. English, D., Roloff, J. S. & Lukens, J. N. *J. Immun.* **126**, 165-171 (1981).
4. Weisbart, R. H., Golde, D. W., Spolter, L., Eggena, P. & Rinderknecht, H. *Clin. Immun. Immunopath.* **14**, 441-448 (1979).
5. Weisbart, R. H. *et al. Clin. Immun. Immunopath.* **22**, 408-418 (1982).
6. Weisbart, R. J. *et al. J. Immun.* **128**, 457-462 (1982).
7. Rosen, H. & Klebanoff, S. J. *J. clin. Invest.* **58**, 50-60 (1976).
8. Wong, G. G. *et al. Science* (in the press).
9. Lopez, A. F. *et al. J. Immun.* **131**, 2983-2988 (1983).
10. Weisbart, R. H. & Mickey, M. R. *J. Immun. Meth.* **16**, 269-281 (1977).
11. Cline, M. J. & Golde, D. W. *Nature* **277**, 177-181 (1979).

Levels of c-myc oncogene mRNA are invariant throughout the cell cycle

Craig B. Thompson*, Peter B. Challoner,
Paul E. Neiman* & Mark Groudine†

Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, Washington 98104, and Departments of *Medicine and †Radiation Oncology, University of Washington, Seattle, Washington 98195, USA

The steady-state messenger RNA levels of several genes increase when cells are stimulated to proliferate¹⁻⁴. The transcripts from one such gene, the proto-oncogene c-myc, increase approximately 20-fold shortly after cells are stimulated to proliferate and then decline before the onset of DNA synthesis^{1,5}. It has been inferred from these data that expression of c-myc may be specific to the G₁ portion of the cell cycle⁶. Alternatively, this transient increase in c-myc mRNA following the stimulation of quiescent cells could be the result of an activational event that renders the cells competent to enter the cell cycle. To distinguish between these possibilities, we performed experiments to determine whether the amount of c-myc mRNA fluctuates during the cell cycle in cells that are under constant stimulation to proliferate. Although c-myc mRNA does undergo a transient increase within 2 h of serum stimulation of quiescent serum-deprived cells, our results show that the level of c-myc mRNA is constant throughout the cell cycle and does not diminish in density-arrested cells maintained in the presence of serum growth factors. In contrast to c-myc, the mRNA levels of two other genes whose expression has been associated with cellular proliferation⁶⁻⁸ do show consistent variations within the cell cycle. Both thymidine kinase (TK) and histone 2b (H2b) mRNA levels increase during S phase in continuously growing cells and decrease when cell replication ceases in density-arrested cultures. Therefore, the transient increase in c-myc transcription following the activation of quiescent cells is not due to the type of cell cycle-dependent regulation characteristic of the TK and H2b genes.

To distinguish events which occur during the activation of quiescent cells to proliferate from events regulated within the cell cycle, we separated cells in exponentially growing cultures by counterflow centrifugation, a technique which fractionates cells primarily on the basis of size^{9,10}. Because cells undergo a linear increase in volume as they progress through the cell cycle⁹, a non-synchronized population of growing cells can be separated by size into subpopulations representing progressive stages of the cell cycle. This method avoids the perturbations in cellular metabolism which are inherent in experiments involving either chemical arrest or deprivation of essential growth factors¹¹. To analyse events within the cell cycle, 10⁸ exponentially growing

cells were loaded into a counterflow centrifuge and separated into six subpopulations of increasing mean cell volume (Fig. 1). To verify that an effective cell cycle separation had occurred, the cells were stained with propidium iodide and the DNA-staining profile analysed on a fluorescence-activated cell sorter (FACS). The average fluorescence per cell of each fraction was calculated and expressed as a multiple of the diploid cell peak. Each fraction was then assigned an average ploidy value ranging from 2n for G₁ cells to 4n for G₂/M cells.

Two types of exponentially growing cell cultures were analysed in this way. MSB-1, a transformed chicken T-cell line with structurally normal c-myc alleles¹², was grown continuously in suspension culture. Chicken embryo fibroblasts (CEF) were dissected from the developing breast muscle of 11-day-old embryos and grown at low density on plastic Petri dishes⁷. Total RNA from cell cycle-dependent subpopulations was isolated using guanidinium isothiocyanate¹³. Ribosomal RNA increased in direct proportion to cell volume as cells progressed from G₁ to G₂/M; because of this, the levels of ribosomal RNA were equalized (Fig. 2) to avoid the measurement of mRNA variations attributable only to increases in cell volume during growth. Northern blots were prepared from the equalized RNA samples and hybridized sequentially with probes specific for the c-myc and TK genes. The blots were also hybridized to probes for the β-actin gene, which encodes an essential structural protein required for cell integrity¹⁴ and therefore should be transcribed in all viable cells, and the gene for H2b, which has been shown previously to be cell cycle-regulated¹⁵. As shown in Fig. 2, the level of c-myc mRNA was equivalent in all of the cell-cycle dependent subpopulations of both CEF and MSB-1 cells. The c-myc mRNA levels as measured by scanning densitometry varied <20% between any two fractions in an individual experiment; this was a consistent finding in several experiments involving both cell types. Levels of β-actin mRNA were also constant in all subpopulations. In contrast, although TK mRNA was present throughout the cell cycle during exponential growth, a two- to sixfold increase in the level of this transcript was found in subpopulations enriched for S-phase cells. Similarly, the H2b mRNA level was 5-15 times greater in the subpopulations containing S-phase cells in both cell types. The quantitative variation in the cell-cycle expression of the H2b gene that we observed was comparable to that reported by others^{15,16}.

As an alternative approach to analysing the association of c-myc expression with cell proliferation, the levels of c-myc mRNA were examined as cells were shifted between exponential growth and growth arrest (Fig. 3a). In the continuous presence of serum growth factors, little difference in the levels of c-myc mRNA was observed between cultures in exponential growth and those that had ceased proliferation. This contrasts with a >10-fold reduction of TK mRNA in both CEF and MSB-1 cells as they stopped proliferating. When quiescent cells were

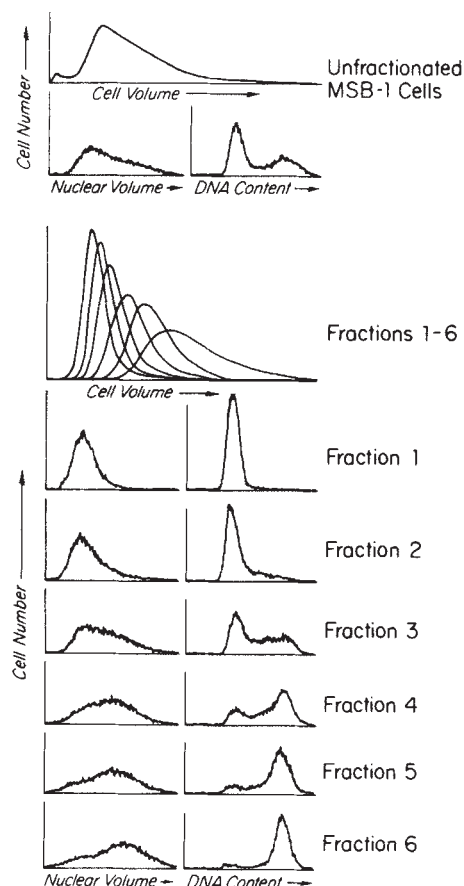


Fig. 1 Representative cell volume, nuclear volume and DNA content analysis of elutriated cell fractions. Exponentially growing MSB-1 cells were separated by counterflow centrifugation (elutriation) into six fractions of increasing cell size which were numbered 1-6 from smallest to largest. The recovery of the unfractionated cells in the six subpopulations was 93%. Cell volume was measured using an electronic cell counter/sizer. Nuclei were prepared by solubilization of cells with 0.5% NP40 in Tris-buffered saline containing 10 mM MgCl₂. Nuclear volume was determined by measuring narrow-angle light scatter on a FACS, and the average DNA content of cells from each fraction was determined by FACS analysis after staining of the nuclei with propidium iodide. n is the haploid DNA content of MSB-1 cells. Unfractionated cells gave a bimodal DNA curve characteristic of exponentially growing cells⁹. The early fractions from the elutriation contained cells with a 2n DNA content characteristic of G₁ cells. As cells of increasing size were recovered, the nuclear volume and average DNA content per cell increased. The final fraction contained almost exclusively cells with 4n DNA content, characteristic of G₂/M cells.

recultured at lower density, both cell types resumed growth and the level of TK mRNA increased, while *c-myc* mRNA remained relatively constant. Again, the H2b mRNA levels paralleled those of TK. Although β -actin mRNA levels were constant in the presence of serum factors in MSB-1 cells, there did seem to be an increase in β -actin mRNA levels during exponential growth of CEF. Similar increases in β -actin mRNA levels have been reported when fibroblasts spread out in subconfluent culture conditions¹⁷.

Figure 3a shows that *c-myc* expression is independent of the proliferative state of a cell. Others have shown that before cells can proliferate they must be activated by growth factors to a state where they are competent to enter the cell cycle¹⁸. Thus, previously described variations in levels of *c-myc* mRNA could be the result of this activation process, rather than the result of regulation during the proliferative cycle. Consistent with the reports of others using murine 3T3 cells^{1,2,4}, we find that serum-deprived cultures of both CEF and MSB-1 avian cells had lower amounts of steady-state *c-myc* mRNA relative to ribosomal

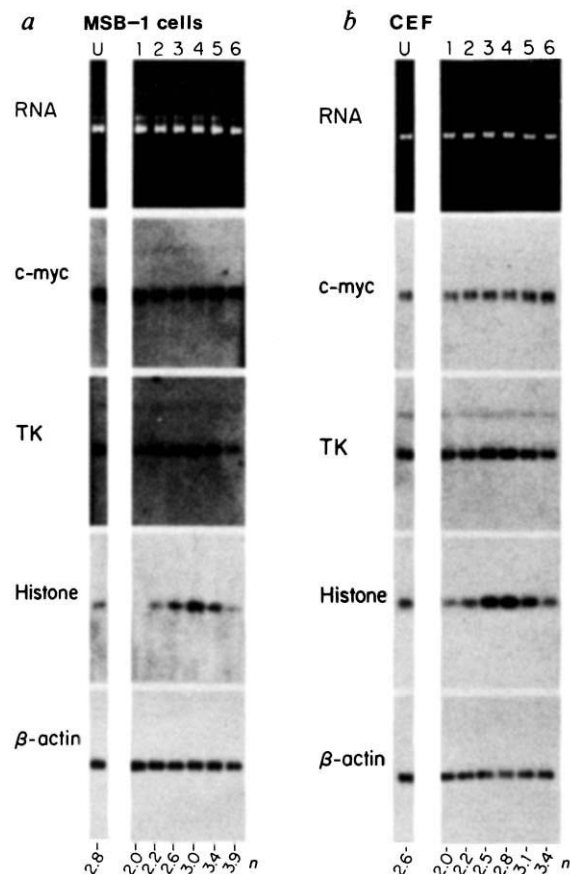
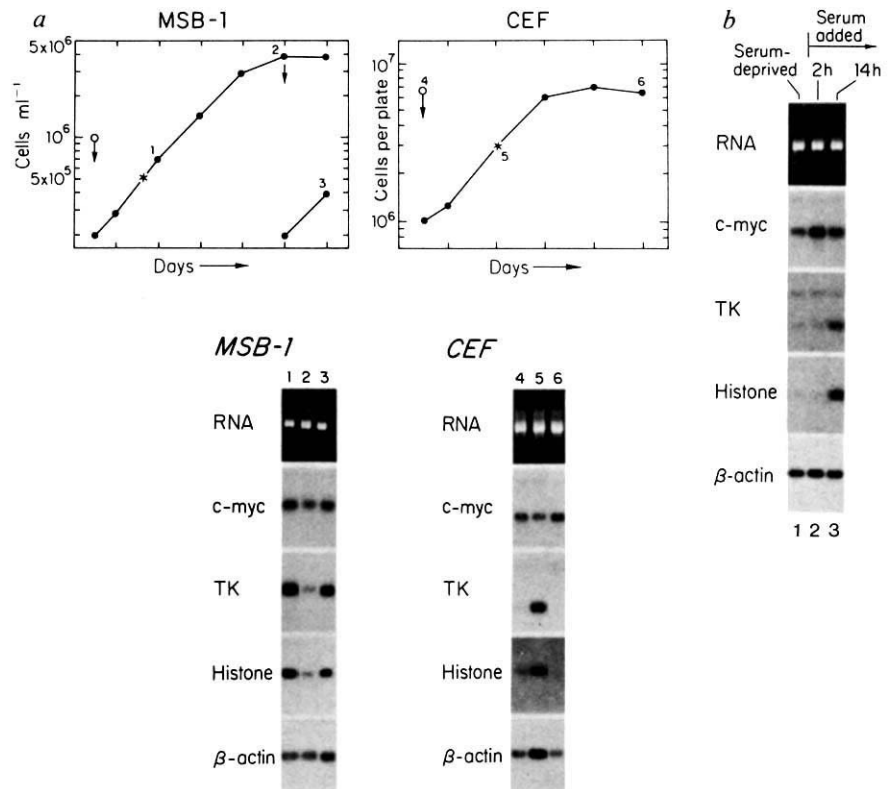


Fig. 2 The *c-myc*, TK, H2b and β -actin mRNA content of cell cycle-separated MSB-1 cells and CEF. Total RNA was prepared from the unfractionated population (U) and each of the subpopulations. The top panels of a and b show ethidium bromide staining of equal amounts of the RNA samples separated on a non-denaturing 1% agarose gel. These equalized RNA samples (2-5 μ g depending on the experiment) were separated on 1% agarose/formaldehyde gels and blotted onto nitrocellulose. Filters were then hybridized with a ³²P-labelled 3.2-kilobase (kb) *Sst*I fragment containing the chicken *c-myc* gene⁷, a 2.9-kb *Hind*III fragment containing a portion of the TK gene⁷ (a gift from M. Wigler), a 0.59-kb *Hind*III fragment containing the 3'-untranslated region of the β -actin gene¹⁴ (a gift from D. W. Cleveland) or a 2.3-kb *Hind*III/*Bam*HI fragment containing the H2b gene²⁴ (a gift of P. A. Krieg). Each filter was probed sequentially with several of these fragments to ensure that the hybridization observed represented true variations between samples rather than variations in sample preparation. Autoradiograms were exposed for 2-48 h and equivalent exposures compared. Relative molecular masses of the mRNAs that hybridized to each of the probes were similar to those published previously^{7,14,24}. The additional band present on some TK and *c-myc* hybridizations was due to nonspecific hybridization to 28S ribosomal RNA and thus provides confirmation that equal amounts of RNA were transferred to the filters. Quantitation was performed by densitometry¹. Numbers below the autoradiograms represent average haploid DNA content (n) of cells in each fraction.

RNA (data not shown). However, the steady-state levels of TK, H2b and β -actin mRNAs also decreased during serum deprivation. Although the cells remained viable and an increase in the levels of *c-myc* was observed on restimulation, it is difficult to be certain that these variations are anything more than a general decrease in steady-state mRNA due to sub-optimal culture conditions. Nevertheless, the response of serum-deprived CEF to the addition of serum growth factors did provide another demonstration of the fact that *c-myc* expression was regulated independently of TK and H2b gene expression. Figure 3b shows an experiment in which confluent CEF were serum-deprived for 3 days and then re-exposed to serum while still confluent. *c-myc* mRNA underwent a transient three- to fivefold increase in

Fig. 3 a, Growth curves of MSB-1 cells and CEF. Exponentially growing MSB-1 cells were followed in culture until no further increase in cell number was observed. They were then recultured at a lower density and resumed exponential growth. Confluent CEF were recultured at a density of 1×10^6 per 150-mm plates and followed to confluency. Below the growth curves are autoradiograms of Northern blots prepared with equal amounts of RNA and hybridized with *c-myc*, TK, H2b and β -actin as described in Fig. 2 legend. For MSB-1, lane 1 represents RNA isolated from cells during initial exponential growth; lane 2, RNA from growth-arrested cells; and lane 3, RNA from cells after resumption of exponential growth. For CEF, lane 4 represents RNA isolated from confluent cells; lane 5, RNA from cells in exponential growth; and lane 6, RNA from cells after they have become confluent again. Cell viabilities as measured by trypan blue exclusion were $>95\%$ at each of the assay points for both CEF and MSB-1 cells. In both cell types, $>40\%$ of the cells were in S or G_2/M phase during exponential growth and $<5\%$ had greater than $2n$ DNA content during growth arrest. $\downarrow$ Indicates the point at which cells were recultured; * is the point on the growth curve at which cells were used for separation into cell cycle-dependent subpopulations using counterflow centrifugation, shown in Fig. 2. **b**, Effect of 3 days' culture of confluent CEF in the presence of medium containing 0.1% serum, followed by stimulation by addition of 6% serum to the culture. Total RNA was prepared before stimulation and 2 h and 14 h after stimulation. Top panel, ethidium bromide-stained gel showing the ribosomal RNAs after equalization; lower panels, autoradiograms of Northern blots of identical 1% agarose/formaldehyde gels hybridized with *c-myc*, TK, H2b and β -actin genes. In each panel, lane 1 is RNA isolated from cells before serum stimulation; lane 2 is RNA isolated from cells 2 h after stimulation; and lane 3 is RNA isolated from cells 14 h after stimulation.



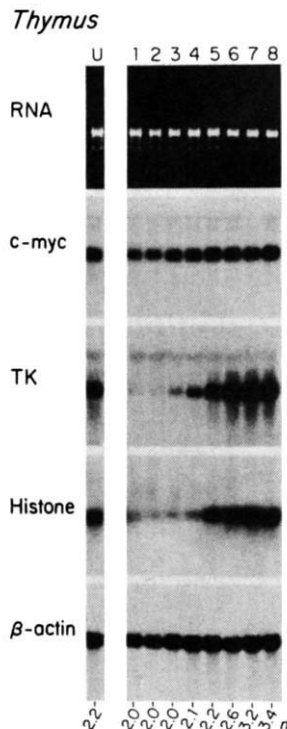
expression 2 h after serum addition and then fell to near quiescent levels by 14 h. The TK and H2b mRNAs were barely detectable either before the addition of serum or 2 h after serum addition, but increased substantially by 14 h, the approximate time required for quiescent CEF to reach S phase following re-exposure to serum factors¹⁸.

Finally, because the conditions in which cells are maintained *in vitro* may be different from the conditions in which cells proliferate *in vivo*, we examined the expression of *c-myc* and TK genes in cell cycle-dependent subpopulations of thymocytes (Fig. 4). Thymocytes proliferating *in vivo* had a wider size range than exponentially growing cells *in vitro*, and the first several subpopulations eluted from the counterflow centrifuge were made up exclusively of cells with $2n$ DNA. The rest of the subpopulations were composed of cells in progressively later stages of the cell cycle, comparable to the subpopulations obtained from CEF and MSB-1 cells. Figure 4 shows that the level of *c-myc* mRNA was slightly lower in the $2n$ thymocyte subpopulations but was constant in all subsequent fractions. The lower *c-myc* mRNA levels in the smaller $2n$ subpopulations correlated closely with the percentage of cells in these subpopulations which had the surface phenotype of mature T cells as assessed by monoclonal antibodies¹⁹ (up to 30% of the cells in the subpopulation containing the smallest cells). Therefore, the $2n$ subpopulations are composed of both thymocytes in the G_1 phase of the cell cycle and small terminally differentiated T cells destined to leave the thymus²⁰. Because terminally differentiated cells have been shown to contain low amounts of *c-myc* mRNA^{4,21}, the low level of *c-myc* mRNA in the $2n$ subpopulations presumably represents the average *c-myc* content of mature T cells and G_1 thymocytes. In contrast to *c-myc*, genes for H2b and TK were expressed only in the subpopulations containing S-phase cells; β -actin mRNA levels were constant in all fractions. Our results suggest that the *in vivo* expression of *c-myc*, H2b, TK and β -actin genes in proliferating thymocyte cells is similar to their expression in cells proliferating *in vitro*.

Previous reports^{1,4,5} have shown that the *c-myc* mRNA levels of murine cells are low in quiescent serum-deprived cells and high in exponentially growing cells. Moreover, growth factors which cause cells to enter the cell cycle have been demonstrated to induce *c-myc* transcription². It has also been shown that following removal of the growth factor responsible for the induction of proliferative competency, the *c-myc* mRNA level rapidly declines, and despite this, cells can proceed through the cell cycle they have already initiated⁵. These results could mean that *c-myc* is a cell-cycle-regulated G_1 -specific transcript⁵. However, for the cells we have examined, the level of *c-myc* mRNA is constant throughout the cell cycle in the continuous presence of serum growth factors. This appears to be a general phenomenon, as we have obtained similar results in a variety of cell types in addition to those presented above, including murine BALB/c 3T3 cells¹, human HL60 cells²¹ and avian BK25 cells¹² (data not shown). As this constant level of *c-myc* mRNA was seen in RNA samples containing equal amounts of ribosomal RNA, and the amount of ribosomal RNA increases as a function of cellular volume as cells progress through the cell cycle, *c-myc* must be transcribed throughout the cell cycle. In the accompanying paper²², data are presented which show that *c-myc* protein is also translated as a fixed percentage of total protein synthesis throughout the cell cycle of both avian and human cells. Based on these results, we postulate that *c-myc* expression reflects the competency of cells to enter and progress through the cell cycle. In the presence of the appropriate growth factors, both proliferative competency and *c-myc* expression can be maintained irrespective of position in the cell cycle.

In contrast to our results with *c-myc*, the TK mRNA level did seem to vary within the cell cycle. TK gene expression has been linked previously to S phase by experiments showing an increase in TK enzymatic activity during DNA replication^{6,23}. Our data demonstrate a similar association between increased TK mRNA levels and S phase. In a pattern of expression that is similar to that for H2b, TK mRNA levels increased during S phase,

Fig. 4 The *c-myc*, TK, H2b and β actin mRNA content of cell cycle separated thymocytes. Numbers at the bottom of the lanes represent the average DNA content per cell (n). Thymocytes were prepared by gently teasing apart the thymuses of 6-week old chickens, and filtering the cell suspension through nylon mesh to remove clumped cells. Contaminating non-lymphoid cells were removed by centrifuging the cell suspension over Ficoll-Hypaque. Greater than 95% of cells prepared in this manner stain positively with monoclonal antibodies specific for thymocyte antigens¹⁹. Cells were separated into subpopulations and analysed in a manner analogous to that shown in Fig. 1 except that two additional subpopulations were isolated at the beginning of the separations due to the wide distribution in size of thymocytes proliferating *in vivo*. Over 50% of the cells in the unfractionated population were isolated in the first three fractions, all of which were exclusively $2n$ in DNA content. Thereafter, the average DNA content and nuclear volume per cell in each fraction increased indicating that a separation on the basis of cell cycle had occurred. Total RNA was prepared from the unfractionated thymocytes (U) of each of the subpopulations. Ribosomal RNA concentrations in all fractions were equalized and ethidium bromide staining of the equalized ribosomal RNAs, separated on a 1% agarose gel, is shown in the upper panel. Autoradiograms of Northern blots were prepared and hybridized with *c-myc*, TK, H2b and β -actin as described in Fig. 2 legend.



7. Groudine, M. & Casimir, C. *Nucleic Acids Res.* **12**, 1427-1446 (1984).
8. Sittman, D. B., Graves, R. A. & Marzluff, W. F. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1849-1853 (1983).
9. Alterman, R. B. M. *et al. Molec. cell. Biol.* **4**, 123-132 (1984).
10. Thompson, C. B. *et al. J. Immun.* **133**, 2333-2342 (1984).
11. Grdina, D. J. *et al. Cell Tissue Kinet.* **17**, 223-236 (1984).
12. Schubach, W. & Groudine, M. *Nature* **307**, 702-708 (1984).
13. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. *Biochemistry* **18**, 5294-5299 (1979).
14. Cleveland, D. W. *et al. Cell* **20**, 95-105 (1980).
15. Osley, M. A. & Hereford, L. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7689-7693 (1982).
16. Zweidler, A. in *Histone Genes: Structure, Organization & Regulation* (eds Stein, G. S., Stein, J. L. & Marzluff, W. F.) 339-371 (Wiley, New York, 1984).
17. Farmer, S. R., Wan, K. M., Ben-Ze'ev, A. & Penman, S. *Molec. cell. Biol.* **3**, 182-189 (1983).
18. Pledger, W. J., Stiles, C. D., Antoniades, H. N. & Scher, C. D. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4481-4485 (1977).
19. Chen, C. H., Chan, T. C. & Cooper, M. D. *Eur. J. Immun.* **14**, 385-391 (1984).
20. Sekaly, R. P., Ceredig, R. & MacDonald, H. R. *J. Immun.* **131**, 1085-1089 (1983).
21. Westin, E. H. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 2490-2494 (1982).
22. Hann, S. R., Thompson, C. B. & Eisenman, R. N. *Nature* **314**, 366-369 (1985).
23. Schlosser, C. A., Steglich, C., DeWet, J. R. & Scheffler, I. E. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1119-1123 (1981).
24. Krieg, P. A. & Melton, D. A. *Nature* **308**, 203-206 (1984).

***c-myc* oncogene protein synthesis is independent of the cell cycle in human and avian cells**

Stephen R. Hann, Craig B. Thompson* & Robert N. Eisenman

Division of Basic Sciences, Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, Washington 98104, USA

* Department of Medicine, University of Washington, Seattle, Washington 98195, USA

Several lines of evidence suggest a role for the *myc* oncogene in cell proliferation.¹⁻⁷ Most recently, mitogenic stimulation of quiescent lymphoid, fibroblast and epithelial cells has been demonstrated to lead to a sharp increase in *c-myc* RNA levels⁸⁻¹⁰. To determine how *c-myc* expression is linked to the cell proliferative cycle, we have used centrifugal elutriation to enrich for populations of avian and human cells at different stages of the cell cycle. Centrifugal elutriation is a counterflow centrifugation method that separates cells on the basis of volume, a parameter correlating well with progression through the cell cycle¹¹⁻¹⁴. Using *myc*-specific anti-peptide antibodies, we show here that the synthesis, half-life and modification of *c-myc* proteins are constant throughout the cell cycle of normal and transformed cells.

Figure 1a (upper panel) shows distributions of cell volume, nuclear volume and DNA content for an exponentially growing population of K562 cells. The K562 line is derived from a human chronic myelogenous leukaemia¹⁵ containing a chromosome 9 translocation involving the *c-abl* oncogene, but does not contain either amplified or translocated *myc* sequences¹⁶. These cells display the broad range of nuclear and cell volumes expected for an unsynchronized population of growing cells. In addition, the DNA content profile indicates the presence of cells in G₁, S and G₂/M phases of the cell cycle. Using centrifugal elutriation of this population of K562 cells, we have collected six subpopulations differing in cell volume (Fig. 1a, lower panel). Flow cytometric analysis of these fractions indicates that fraction 1 has a DNA content characteristic of cells in G₁, fractions 2 and 3 contain cells in G₁ with increasing numbers of S-phase cells, fractions 4 and 5 contain primarily S-phase cells with some G₁ and G₂/M cells, respectively, and fraction 6 contains predominantly G₂/M cells. The increase in nuclear and cellular volume observed during progression through the cell cycle is paralleled by an increase in both the quantity of ribosomal RNA and amino-acid incorporation, to levels in G₂/M cells more than twice those found in G₁ cells (data not shown).

To determine the level of *c-myc*-encoded protein synthesis, K562 cells were pulse-labelled for 20 min with ³⁵S-methionine before elutriation. The labelled cells from each elutriator fraction were lysed and sonicated in buffer containing detergents. Because incorporation of the labelled amino acid increased in direct proportion to cell volume during progression through the

decreased as the number of replicating cells declined in culture, and was not expressed until 14 h after serum stimulation of quiescent cells. However, the variation of TK mRNA levels within the cell cycle of exponentially growing cells was not as large as the variation of H2b mRNA. There are several possible reasons for this. Unlike the H2b gene, expression of the TK gene may be only partially S-phase-dependent. For example, histone variants have been described in which transcription is induced at the start of DNA synthesis but is not completely repressed following S phase¹⁶. Alternatively, the half-life of TK mRNA may be greater than that of H2b, possibly because of the lack of polyadenylation of histone mRNA⁸.

Taken together, these data define two broad categories of mRNA expression with respect to the cell cycle. Some genes, such as those for TK and H2b, are expressed at specific points in the cell cycle and can be said to be cell-cycle-regulated. A second class of genes, represented by *c-myc*, are expressed in cells capable of proliferating and are transcribed continuously throughout the cell cycle.

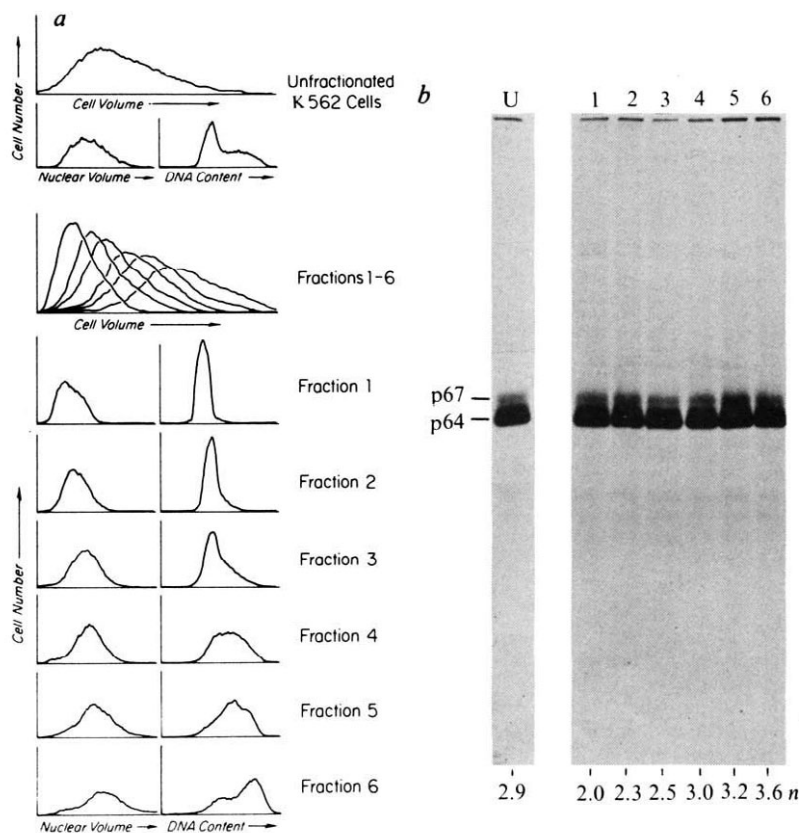
We thank our colleagues Kathleen Conklin, William Forrester, Stephen Hann and Robert Eisenman for stimulating discussions and critical comments on the manuscript and Rainer Storb for use of his counterflow centrifugation equipment. This investigation was supported by grant CA 20068 from the National Cancer Institute, DHHS, and grant PCM 82-04696 from the NSF. P.B.C. is supported by NIH fellowship, 1 F32 HD06583-01. M.G. is a Scholar of the Leukemia Society of America.

Received 29 November 1984; accepted 19 February 1985.

1. Kelly, K., Cochran, B. H., Stiles, C. D. & Leder, P. *Cell* **35**, 603-610 (1983).
2. Greenberg, M. E. & Ziff, E. B. *Nature* **311**, 433-438 (1984).
3. Goyette, M., Petropoulos, C. J., Shank, P. R. & Fausto, N. *Molec. cell. Biol.* **4**, 1493-1498 (1984).
4. Campisi, J. *et al. Cell* **36**, 241-247 (1984).
5. Kelly, K., Cochran, B., Stiles, C. & Leder, P. *Curr. Topics Microbiol. Immun.* **113**, 117-126 (1984).
6. Johnson, L. F., Rao, L. G. & Muench, A. J. *Exp. Cell Res.* **138**, 79-85 (1982).

Fig. 1 *a*, Representative cell volume, nuclear volume and DNA content analysis of elutriated cell fractions. *b*, Analysis of *c-myc*-encoded proteins in cell cycle-separated K562 cells. *N* represents the haploid DNA content of K562 cells; U, unfractionated population.

Methods. *a*, An exponentially growing culture of the human chronic myelogenous leukaemia cell line K562 was suspended in Tris-buffered saline with 0.5 mM EDTA, 5% calf serum at 4 °C and separated by counterflow centrifugation into six fractions numbered 1–6 in order of increasing cell size. The recovery of the input unfractionated cells from the six subpopulations was 90%. Cell volume was measured using an electronic cell counter/sizer. Nuclear volume was determined by measuring narrow angle laser light scatter on a fluorescence-activated cell sorter (FACS) after preparation of nuclei by solubilization of cells with 0.5% NP40 in Tris-buffered saline containing 10 mM MgCl₂. The average DNA content of cells from each fraction was determined by FACS analysis after staining the nuclei with propidium iodide. *b*, The K562 cells (10⁸ cells) shown in the elutriation fractions of *a* were first labelled with 3 mCi ³⁵S-methionine before elutriation. The elutriated subpopulations were lysed in Ab. buffer (0.01 M Tris-Cl pH 7.5, 0.05 M NaCl, 0.5% NP40, 0.5% deoxycholate, 0.5% SDS, 0.5% Trasylol, 1 mM phenylmethylsulphonyl fluoride, 10 mM iodoacetamide), followed by 30 s of sonication and centrifugation at 10,000g for 10 min. The lysates were adjusted to equal levels of incorporated label and brought to the same volume. Immunoprecipitation was as described previously using anti-hu-*c-myc* 12C (affinity-purified antibodies prepared against a synthetic peptide whose sequence corresponds to the 12 C-terminal amino acids of the human *c-myc* coding region)¹⁷. The immunoprecipitate was dissolved by boiling for 3 min in SDS electrophoresis sample buffer (5% SDS, 4% β-mercaptoethanol, 0.08 M Tris-Cl pH 6.8, 10% glycerol, 0.002% bromophenol blue) and electrophoresed on a discontinuous 10% SDS-polyacrylamide slab gel. A fluorograph of the dried gel is shown. *M_s* were determined as previously described¹⁷; *M_r* markers were co-electrophoresed on all gels. Quantitation was carried out by scanning laser densitometry of fluorographs.



cell cycle (data not shown), we adjusted the amounts of lysate to normalize for equal amounts of incorporated label¹⁷. This allows an analysis of changes in *c-myc* protein levels that are not due simply to changes in cell mass during growth. The *c-myc* proteins were immunoprecipitated with an anti-peptide antibody reactive with the carboxy-terminus of human *c-myc* proteins (anti-hu-*c-myc* 12C)¹⁷. Figure 1*b* demonstrates that K562 cells synthesize the major and minor human *c-myc*-specific proteins p64 and p67 (relative molecular masses (*M_s*) 64,000 and 67,000) as previously observed for cells with unarranged *c-myc*¹⁷ and also that the levels of p64/p67^{myc} are constant (<25% maximum variation among the fractions) in the six cell fractions derived from different phases of the cell cycle. The short labelling time used in these experiments (20 min) coupled with the rapid elutriation time (45 min) at 4 °C implies that we are measuring primarily the rate of synthesis of *myc* proteins, which have a half-life of ~25 min at 37 °C¹⁷. Similar results to those shown in Fig. 1*b* were obtained when cell populations were labelled after elutriation (data not shown), indicating that the elutriation procedure is unlikely to be perturbing *myc* protein metabolism.

To find whether production of *c-myc* protein is different in transformed cells containing an altered *c-myc* allele, we also examined Manca, a human Burkitt's lymphoma cell line, which contains a translocation (t(8; 14)) of *c-myc* exons 2 and 3 into the immunoglobulin heavy-chain locus^{18,19} and produces only the p64^{myc} protein¹⁷ (see Fig. 2*a*, lane U). When *c-myc* protein synthesis in Manca cells was assayed after fractionation by elutriation into cell cycle stages, as seen with K562 cells, the rate of synthesis of p64^{myc} showed little variation (23% among the average G₁, S and G₂/M subpopulations) throughout the growth cycle when cell extracts were normalized to equal amounts of incorporated label (Fig. 2*a*).

We next attempted to generalize these observations by examining *c-myc* protein synthesis as a function of the cell cycle in normal and transformed avian cells. Accordingly, we labelled with ³⁵S-methionine: (1) secondary quail embryo fibroblast cells (QEF), (2) a Marek's disease virus-transformed chicken thymocyte line (MSB-1 cells) containing unarranged *c-myc* (ref. 20) and (3) the chicken bursal lymphoma cell line BK25, containing a *c-myc* gene activated by insertion of a long terminal repeat²¹. The labelled cells were fractionated by elutriation, the lysates adjusted to equal amounts of incorporated label and avian *c-myc* proteins identified by immunoprecipitation with anti-peptide antiserum prepared against the carboxy-terminus of the MC29 v-*c-myc*-encoded protein (anti-v-*c-myc* 12C)²². The QEF (Fig. 2*b*) and MSB-1 cells (Fig. 2*c*) showed the avian p59/62^{c-myc} proteins characteristic of cells possessing unaltered *c-myc* genes, while the BK25 bursal lymphoma line (Fig. 2*d*) showed high levels of only one of the *c-myc* proteins (p59^{myc}), similar to that previously reported for human tumour cells containing *c-myc* alterations¹⁷. The data again demonstrate no consistent change in the levels of synthesis of these *c-myc*-encoded proteins. A maximum of 20% variation was found among the BK25 fractions, with 30% variation among the MSB-1 fractions.

We also assayed for *c-myc* protein synthesis in a synchronized QEF population at various times after release from a double-thymidine block at the G₁/S boundary. Cells released into serum-containing medium doubled in cell number ~10 h after release, but levels of *c-myc* protein synthesis, except for a transient increase 1 h after release, did not change significantly throughout the 24-h QEF cell cycle (Fig. 2*e*). In addition, the G₁/S-blocked non-proliferating population had levels of *c-myc* protein synthesis equal to that of the mock-treated proliferating cells (compare Fig. 2*e*, lanes 0 and M). In a parallel experiment,

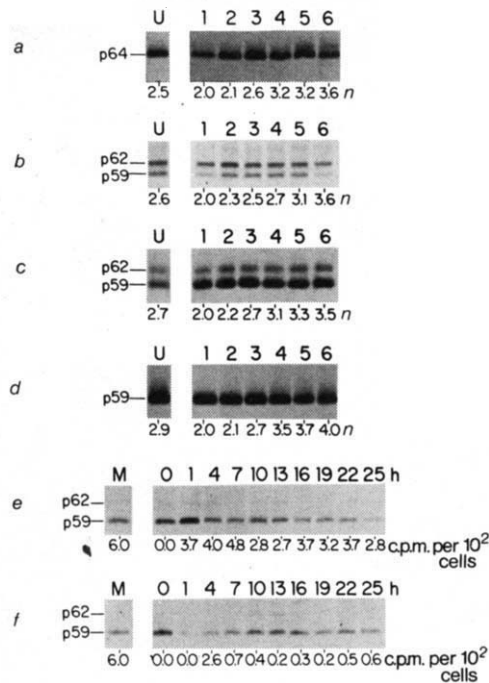


Fig. 2 *a-d*, Synthesis of *c-myc* proteins in human and avian cells separated into cell cycle subpopulations. Exponentially growing cells (10^8) were labelled for 20 min with 1–3 mCi ^{35}S -methionine before elutriation. Lysates from cell cycle subpopulations were adjusted to equal amounts of incorporated label and immunoprecipitated (*a*, anti-hu-*myc* 12C; *b-d*, anti-v-*myc* 12C (ref. 22)) as described in Fig. 1 legend. *a*, Manca cell line, a human Burkitt's lymphoma cell line^{18,19}; *b*, quail embryo fibroblast cells, secondary culture; *c*, MSB-1 cells, a chicken thymocyte line; *d*, BK25, a chicken cell line derived from a bursal lymphoma. Numbers below the autoradiographs represent average DNA content of cells in each fraction. U, unfractionated cells. *e, f*, Synthesis of *c-myc* proteins in chicken embryo fibroblast cells synchronized by double-thymidine block. Secondary cultures of chicken embryo fibroblasts (CEF) were grown in 100-mm dishes to 5×10^6 cells per dish and treated with 25 mM thymidine for 18 h. This was followed by growth in complete medium for 9 h and another 18-h incubation in complete medium containing 25 mM thymidine. Cells were labelled with 500 μCi ^{35}S -methionine per ml for 20 min before removal of the thymidine (0 time point) and at 3-h intervals after removal of the thymidine and incubation in growth medium containing 10% calf serum, 2% chick serum (*e*) or in growth medium lacking serum (*f*). Preparation of lysates and immunoprecipitation with anti-v-*myc* 12C were as described in Fig. 1 legend. Parallel cultures were labelled for 20 min with 5 μCi ^3H -thymidine per ml and aliquots of lysates were precipitated with trichloroacetic acid to determine incorporation of label into DNA as denoted by numbers below the lanes. Synchrony induction was confirmed by direct cell counting. Lanes M, mock-treated cultures. Only the relevant portions of the autoradiographs are shown. Lower band intensities in *e* (25 h) and *f* (1 h) lanes were due to sample loss during preparation of immunoprecipitates.

we measured *c-myc* protein synthesis in QEF released from the double-thymidine block into serum-free medium (Fig. 2*f*). In contrast to the cells released into complete medium, these cells showed only a small increase in incorporated ^3H -thymidine label at 4 h after release, no cell division up to 25 h after release and a significant decrease in ^{35}S -methionine incorporation. Figure 2*f* shows that these arrested quiescent cells had virtually the same levels of *c-myc* protein synthesis as the actively growing cells released into complete medium throughout most of the 25-h period (compare Fig. 2*e* and *f*). The transient changes in *c-myc* protein synthesis observed 1 h after release would seem to be a serum-dependent effect on synthesis rather than a cell cycle-regulated event.

To relate the levels of *c-myc* protein synthesis observed during the cell cycle of proliferating cells to the levels found in non-proliferating cells arrested in G_1 , we sought to determine whether

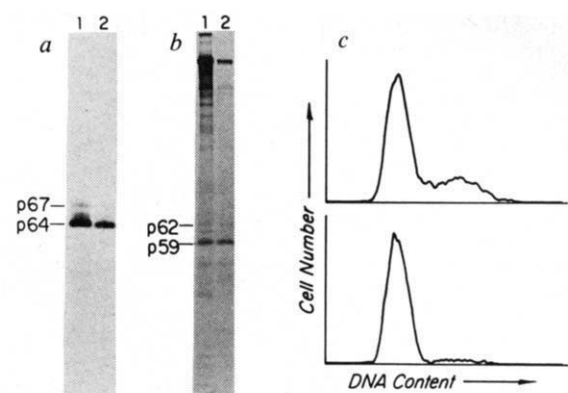


Fig. 3 Synthesis of *c-myc* proteins in quiescent and growing human and avian cells. *a*, K562 cells; *b*, QEF. Lanes 1, exponentially growing cells; lanes 2, quiescent cells. *c*, FACS analysis of the DNA contents of QEF cells in *b*. Upper panel, exponentially growing cells; lower panel, quiescent cells.

Methods. Human K562 cells were grown to saturation (10^6 cells per ml) and tertiary cultures of QEF were grown to confluency (2×10^7 cells per 100-mm dish) and incubated in normal growth medium for an additional 48 h. The cells were then incubated for a further 48 h in medium containing 0.1% serum (quiescent cells) and then labelled for 20 min with 500 μCi ^{35}S -methionine in medium lacking serum. Parallel cultures of non-serum-starved exponentially growing cells ($4 \times 10^5 \text{ ml}^{-1}$ K562 cells; 10^7 per 100-mm dish QEF) were also maintained and labelled at the same time. FACS analysis indicated the presence of G_1 , S and G_2/M cells in the exponentially growing populations. Lysis and immunoprecipitation with anti-v-*myc* 12C were as described in Fig. 1 legend.

Fig. 4 Two-dimensional gel electrophoretic analysis of *c-myc* proteins synthesized in cell cycle-separated subpopulations of MSB-1 cells. Numbers on the left-hand side refer to the cell fractions; U, unfractionated cells; *n* represents the haploid DNA content.

Methods. Chicken MSB-1 cells (10^8 cells) were labelled for 20 min with 3 mCi ^{35}S -methionine before centrifugal elutriation. The isolated subpopulations were lysed and immunoprecipitated with anti-v-*myc* 12C as previously described except that the final washed immune complex was dissolved by boiling for 1 min in 9.5 M urea, 0.5% SDS, 5% β -mercaptoethanol, 0.2% ampholines pH 3.5–10 followed by addition of an equal volume of 9.5 M urea, 2% w/v NP40, 1.6% ampholines pH 5.0–7.0, 0.4% ampholines pH 3.5–10.0, 5% β -mercaptoethanol and boiling for 1 min. Equilibrium isoelectric focusing was carried out as described by O'Farrell²⁵.

Briefly, the solubilized immune complex was applied to a first-dimension equilibrium isoelectric focusing tube gel. Following electrophoresis to equilibrium, the gel was removed, equilibrated in 10% glycerol, 5% β -mercaptoethanol, 2.3% SDS, 62.5 mM Tris-Cl pH 6.8, and electrophoresed in the orthogonal direction on 10% SDS-polyacrylamide slab gels. Only the relevant portions of the second-dimension gels are shown. The pH gradients in the isoelectric focusing gels were determined by incubating 5-mm slices of a parallel gel in degassed distilled water and measuring the pH of the eluted ampholine.

the levels of *c-myc* protein differed between quiescent G_1 cells deprived of serum and cells growing in the presence of serum. To obtain quiescent cells, we grew human K562 cells to saturation, and QEF to confluency, in serum-containing medium for 48 h followed by a further 48-h incubation in medium with 0.1% serum. The serum-starved K562 cells showed a 10-fold less ^3H -thymidine uptake than the parallel culture of proliferating cells. Both populations showed substantial *c-myc* protein syn-

thesis, although the proliferating cells had twice the level of *c-myc* protein (Fig. 3a). The QEF also showed a decrease in ³H-thymidine incorporation. Cytofluorometric measurement of the DNA content of the serum-starved QEF showed a complete absence of S and G₂/M cells, in contrast to the non-serum-starved cells which showed the expected levels of S and G₂/M cells (Fig. 3c). Figure 3b shows that there is no significant difference in *c-myc* protein levels in proliferating and non-proliferating QEF.

Although the synthesis of *c-myc* proteins seems not to change significantly during the cell cycle, two other properties relating to *c-myc* proteins, turnover and post-translational modification, could potentially vary in a cell cycle-dependent manner. Pulse-chase experiments indicate an extremely short apparent half-life (20–30 min) for avian *v-myc* and human *c-myc* proteins^{17,23}. We therefore used this technique on chicken MSB-1 cells separated by centrifugal elutriation. After a 15-min pulse and a 60-min chase, there was no significant change in apparent turnover of *c-myc* protein as a function of the cell cycle (data not shown).

To examine post-translational modification of *c-myc* protein as a function of the cell cycle, we performed two-dimensional gel electrophoretic analyses. The only known modification of *myc* proteins is phosphorylation^{17,22,24}. Changes in the extent of phosphorylation, or some other type of charge modification, would probably produce charge alterations that would be detectable as shifts in isoelectric point²⁵. MSB-1 cells labelled with ³⁵S-methionine for 20 min were separated by elutriation, lysed and the anti-*v-myc* 12C immunoprecipitate dissolved and subjected to equilibrium isoelectric focusing in the horizontal dimension and SDS-polyacrylamide gel electrophoresis in the vertical dimension²⁵. Figure 4 shows that the avian p59^{myc} protein is resolved into a major spot of pI 6.0 which trails into a poorly resolved minor spot of pI 5.8. The minor p62^{myc} protein and another faint spot also have pIs of 6.0, suggesting that the difference between p59^{myc} and the higher-*M_r* forms is not due to hyperphosphorylation or other charge-altering modifications. Comparison of the two-dimensional electrophoretic patterns of the *c-myc* proteins in MSB-1 cells separated into different cell cycle subpopulations revealed no alterations in isoelectric point (Fig. 4). Finally, we have also failed to detect differences in ³²P-labelling of *c-myc* proteins at different times after release of MSB-1 cells from a G₁/S block (data not shown).

Our results demonstrate that in avian and human cells the rate of synthesis (as a fraction of total cell protein synthesis), turnover and modification of *c-myc* proteins is independent of the cell cycle stage. Several reasons make it unlikely that we did not achieve an effective separation of cells into different cell cycle stages. The increases observed in cell volume, nuclear volume and DNA content (Fig. 1a) and in protein synthesis and ribosomal RNA content in our isolated subpopulations all correlate well with progression through the cell cycle^{11,12}. Furthermore, the accompanying manuscript shows that the levels of *c-myc* RNA in avian cells are also constant throughout the cell cycle, even though levels of histone 2b and thymidine kinase messenger RNAs vary during the cell cycle in the same cells²⁶. Previous reports have indicated that stimulation of quiescent cells with growth factor results in a rapid increase in *c-myc* transcription⁸. One interpretation of these previous findings has been that *c-myc* is 'switched on' during the transition from a G₀ to a proliferating state and that high levels of *c-myc* expression may be needed to traverse each G₁ period^{27,28}. However, the data in this and the accompanying paper demonstrate that the levels of *c-myc* expression are essentially the same in all growth phases of proliferating cell populations and in non-proliferating cells arrested in G₁ or G₁/S. We conclude that the characteristic mode of *c-myc* expression is continuous synthesis and rapid turnover throughout the cell cycle in non-terminally differentiated cells and tumour cells. The increase in *c-myc* expression on stimulation of resting cells is only transient, with the elevated levels of both *c-myc* RNA^{8,10} and protein²⁹ falling back to a constant basal level which is then maintained throughout subsequent cell cycles.

We thank Susan Fisher for technical assistance and Mark Groudine, Peter Challoner, Paul Neiman and Maxine Linial for stimulating discussions and critical readings of the manuscript. This work was supported by NCI grants CA 20525 and CA 2851 to R.N.E. S.R.H. is a Special Fellow of the Leukemia Society of America.

Received 29 November 1984; accepted 19 February 1985.

1. Royer-Pokora, B. *et al.* *Cell* **13**, 751–760 (1978).
2. Palmieri, S., Kahn, P. & Graf, T. *EMBO J.* **2**, 2385–2389 (1983).
3. Land, H., Parada, L. F. & Weinberg, R. A. *Science* **222**, 771–778 (1983).
4. Campisi, J., Gray, H. E., Pardee, A. B., Dean, M. & Sonenshein, G. E. *Cell* **36**, 241–247 (1984).
5. Gonda, T. J. & Metcalf, D. *Nature* **310**, 249–251 (1984).
6. Lachman, H. M. & Skoultschi, A. I. *Nature* **310**, 592–594 (1984).
7. Westin, E. H. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **79**, 2490–2494 (1982).
8. Kelly, K., Cochran, B. H., Stiles, C. D. & Leder, P. *Cell* **35**, 603–610 (1983).
9. Makino, R., Hayashi, K. & Sugimura, T. *Nature* **310**, 697–698 (1984).
10. Greenberg, M. E. & Ziff, E. B. *Nature* **311**, 433–438 (1984).
11. Killander, D. & Zetterberg, A. *Expl Cell Res.* **38**, 272–284 (1965).
12. Zetterberg, A. *Expl Cell Res.* **42**, 500–513 (1966).
13. Alterman, R. B. M. *et al.* *Molec. cell. Biol.* **4**, 123–132 (1984).
14. Thompson, C. B. *et al.* *J. Immun.* **133**, 2333–2342 (1984).
15. Lozzio, C. B. & Lozzio, B. B. *Blood* **45**, 321–344 (1975).
16. Collins, S. J. & Groudine, M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4813–4817 (1983).
17. Hann, S. R. & Eisenman, R. N. *Molec. cell. Biol.* **4**, 2486–2497 (1984).
18. Taub, R. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **79**, 7837–7841 (1982).
19. Neel, B., Jhanwar, S., Chaganti, R. & Hayward, W. S. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7842–7846 (1982).
20. Schubach, W. & Groudine, M. *Nature* **307**, 702–708 (1984).
21. Pahl, C., Schubach, W., Eisenman, R. & Linial, M. *Cell* **33**, 335–344 (1983).
22. Hann, S. R., Abrams, H. D., Rohrschneider, L. R. & Eisenman, R. N. *Cell* **34**, 789–798 (1983).
23. Eisenman, R. *et al.* *Cold Spring Harb. Symp. quant. Biol.* **44**, 1215–1223 (1980).
24. Ramsay, G., Hayman, M. J. & Bister, K. *EMBO J.* **1**, 1111–1116 (1982).
25. O'Farrell, P. H. *J. biol. Chem.* **250**, 4007–4021 (1975).
26. Thompson, C. B., Challoner, P. B., Neiman, P. E. & Groudine, M. *Nature this issue*
27. Kelly, K., Cochran, B., Stiles, C. & Leder, P. *Curr. Topics Microbiol. Immun.* **113**, 117–126 (1984).
28. Pardee, A. B., Campisi, J., Gray, H. E., Dean, M. & Sonenshein, G. in *Mediators in Cell Growth and Differentiation* (eds Ford, R. J. & Maizel, A. L.) 21–29 (Raven, New York, 1985).
29. Persson, H., Henninghausen, L., Taub, R., DeGrado, W. & Leder, P. *Science* **225**, 687–693 (1984).

Isolation of a cDNA clone corresponding to an X-linked gene family (XLR) closely linked to the murine immunodeficiency disorder *xid*

David I. Cohen*†, Stephen M. Hedrick†¶, Ellen A. Nielsen†, Peter D'Eustachio‡, Frank Ruddle§, Alfred D. Steinberg||, William E. Paul† & Mark M. Davis*†

* Department of Medical Microbiology, Stanford University School of Medicine, Stanford, California 94305, USA

† Laboratory of Immunology, NIAID, National Institutes of Health, Bethesda, Maryland 20205, USA

‡ Department of Biochemistry, New York University Medical Center, New York, New York 10016, USA

§ Department of Biology, Yale University, New Haven, Connecticut 06511, USA

|| Arthritis and Rheumatism Branch, NIADDK, NIH, Bethesda, Maryland 20205, USA

The striking number of human and murine immunodeficiency disorders which map to the X chromosome (Table 1) suggests that genes localized on this chromosome must have important roles in lymphocyte development. At least seven distinct disorders in the human and two in the mouse disrupt lymphocyte maturation, particularly that of B cells, at characteristic stages^{1,2}. As functional genes mapping to the X chromosome in one mammal are found on the X chromosome in all other mammals³, the same genes regulating lymphocyte development are expected to be found on the X chromosome in mouse and man. Investigations into the possible mechanisms of these X-linked disorders have been hampered by the lack of molecular probes for the genes or gene products affected; because of this, and the possibility of correlating

¶ Present address: Department of Biology, M-001, University of California at San Diego, La Jolla, California 92093, USA.

one or more of the several hundred B- or T-cell-specific genes⁴ with a specific mutation, we surveyed 15 different B- and T-cell-specific cDNA clones for localization to the X chromosome. We report here the characterization of one of these murine cDNA clones, which hybridizes with a large, X-linked gene family, designated XLR (X-linked, lymphocyte-regulated). We show that the XLR gene family is closely linked to the X-linked immunodeficiency described in the CBA/N mouse strain (*xid*)⁵, by restriction fragment length polymorphism (RFLP) analysis of DNA from mice congenic for *xid*. This finding, together with data on the expression of the XLR locus in B cells (see accompanying paper⁶), indicates that this gene family either includes the locus defined by the *xid* mutation or is adjacent to it in a gene complex which may be important in lymphocyte differentiation.

The initial cDNA clone, pX310, which identified XLR was obtained by screening a T-cell-specific, selected cDNA library⁷ with a T-helper cDNA-T-suppressor mRNA-subtracted cDNA probe. Although pX310 was originally isolated in this manner as a T-cell-specific clone, subsequent analysis shows that mRNAs which cross-hybridize with this cDNA are also expressed in late-stage and secretory B-lymphocyte tumour lines, but not in the original B-cell tumour (BAL 17) used in the subtraction of the T-cell library (see accompanying report⁶). pX310 was one of 15 clones, obtained from T-cell-specific or B-cell-specific libraries, which were analysed by Southern blotting to mouse-hamster somatic cell hybrids⁸. This cDNA clone characteristically hybridizes with 10–14 bands on Southern analysis, consistent with its recognizing a gene family, and *Eco*RI restriction digests of DNAs from the somatic cell hybrid lines indicate that all of the restriction fragments which hybridize to pX310 are found on the mouse X chromosome (Fig. 1). Most useful for such a chromosomal assignment are results obtained from an analysis of the somatic cell hybrids MAE 28, containing the murine chromosomes 12 and X; and MAE 32, containing chromosomes 16 and X; and MACH 4A63, containing most of the mouse chromosomes, including chromosomes 12 and 16 but not X (see Fig. 1). DNAs from the former two cell lines hybridize with pX310, while DNA from the latter line does not. None of 14 other cDNA clones obtained from the T- or B-cell-enriched cDNA libraries show evidence of being derived principally from X-chromosomal genes, or of recognizing such a large gene family. That the multiple bands seen with the pX310 probe are not due to 5'- or 3'-untranslated region repeats is indicated by the fact that an internal restriction fragment from an apparently full-length XLR cDNA clone gives an identical pattern on Southern blots (data not shown).

The 10–14 bands seen on Southern analysis of pX310 are comparable to the 15–28 bands seen with murine class I major histocompatibility complex (MHC) gene probes^{9,10} (corresponding to ~35 different genes¹¹) and to the 8–10 bands seen for α -interferon (IFN- α) corresponding to ~10 distinct genes¹².

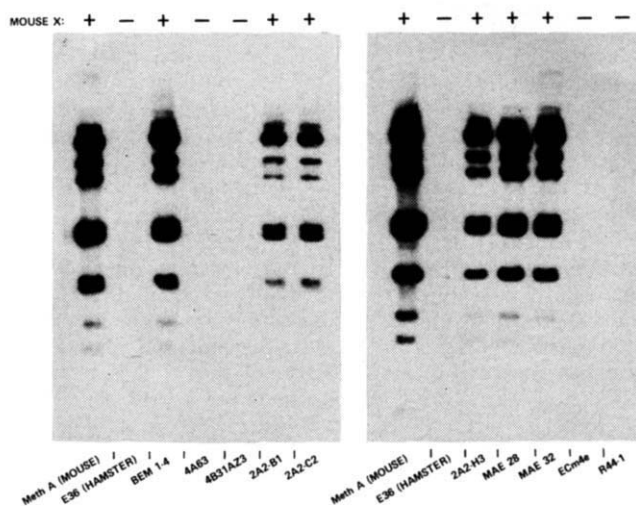


Fig. 1 X-linkage of the XLR gene family. DNAs were isolated from mouse-hamster somatic cell hybrids, digested with the restriction enzyme *Eco*RI, and blotted onto nitrocellulose according to the method of Southern⁸. The insert from XLR cDNA clone pM1 was labelled by nick-translation to 5×10^8 c.p.m. μg^{-1} , blots were hybridized with 10^6 c.p.m. ml^{-1} in $5 \times \text{SSPE}^{28}$, 50% formamide at 42°C , and washed repeatedly before exposure, the most stringent wash being in $0.2 \times \text{SSPE}$ at 60°C . The names of hybrid lines are given at the bottom, and the presence (+) or absence (-) of the mouse X chromosome in each hybrid is indicated above each lane. The chromosomal content of each line has been described elsewhere^{29,30}, and the copies of mouse chromosome X per hybrid cell are: meth A (2.0), E36 (0), BEM 1-4 (0.87), 4A63 (0), 4B31A23 (0), 2A2-B1 (0.35), 2A2-C2 (0.60), 2A2-H3 (0.70), MAE 28 (1.00), MAE 32 (1.00), ECm4e (0), and R44-1 (0).

Similar to the class I MHC genes, there are no apparent dispersed members on other chromosomes. While an XLR probe gives little or no cross-hybridization with hamster DNA in moderately stringent conditions (see Fig. 1 legend), it does hybridize well with DNAs from three distinct wild mouse species and, indeed, shows less polymorphism than another gene family, PGK, for these DNAs (D.I.C. and M.M.D., unpublished data). This is distinctly different from the pattern seen for mobile elements¹³. Both class I MHC and IFN- α genes are genetically linked in a cluster to other very distantly related genes; in the former case, to the class II MHC genes¹⁴, and in the latter case, to β -interferon¹⁵.

One of the most studied X-chromosomal defects is the *xid* lesion^{16,17}, which causes a recessive B-cell immunodeficiency which appears to disrupt late-stage B-cell functions¹⁸. The mutation was recognized in a subline of mice, designated CBA/N,

Table 1 X-linked immunodeficiency diseases

Name of defect	Cell types affected	Presumed stage	Functional defect
Human defects (ref. 1)			
Severe combined immunodeficiency	T, B	Stem cell	T-cell immunity (TCI), antibody (Ab)
Wiskott-Aldrich syndrome	T, B, platelets	Haematopoietic	TCI, Ab to polysaccharides, low platelets
Agammaglobulinaemia (Bruton's)	B	Pre-B cell	Ab
Agammaglobulinaemia with growth hormone deficiency	B	Pre-B cell	Ab
Lymphoproliferative syndrome	B	B-cell growth regulation	Lymphomas on Epstein-Barr virus infection
Hyper IgM, reduced IgG and IgA	B	Plasma cells	Low IgG, low IgA
Hyper IgM and IgG, reduced IgA	B	Plasma cells	Low IgA
Murine defects (refs 2, 19, 20)			
CBA/N (<i>xid</i>)	B	Mature B cell	Antibody to polysaccharides
DBA/2Ha	B	Mature B cell	Plasma cell differentiation

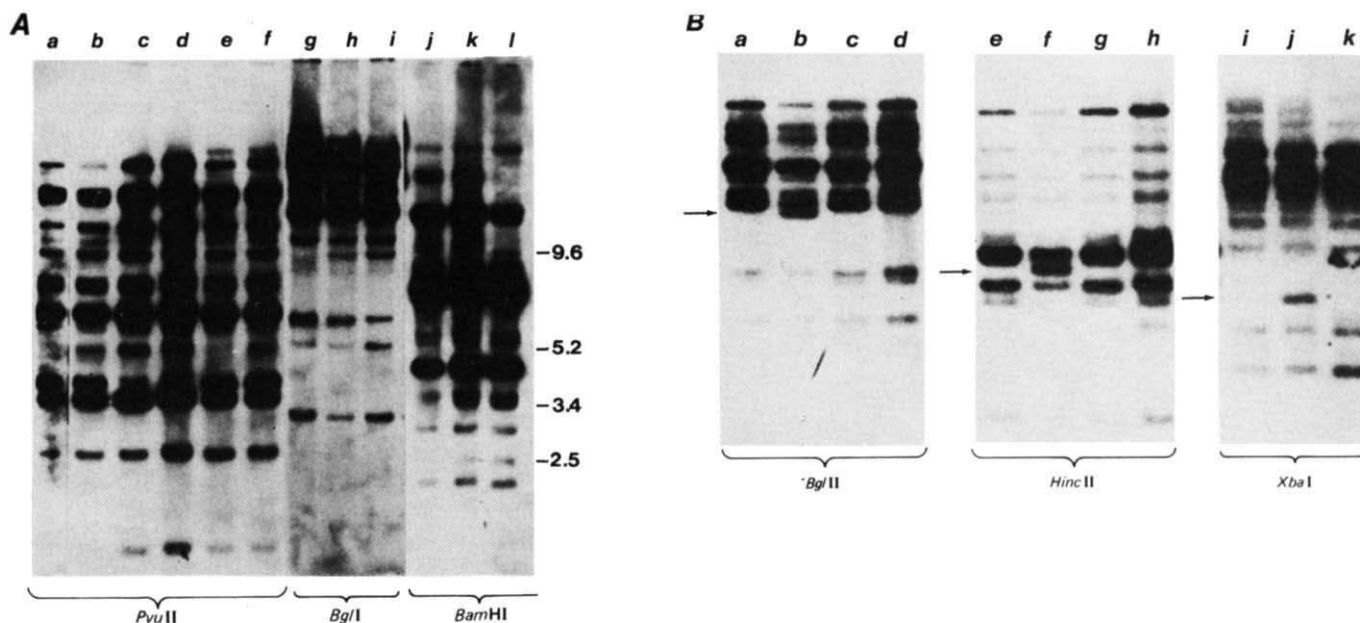


Fig. 2 A, Restriction fragment length polymorphism analysis of genomic DNA from MRL (lanes *a, g*), CBA/N (*c, i, l*), CBA/Ca (*d*), BXSB (*e, j*), and from the *xid* congenic strains, MRL *xid* (*b, h*) and BXSB *xid* (*f, k*). Polymorphisms referred to in the text are labelled by size (in kb) on the right of the figure; restriction enzymes are indicated at the bottom. Blots were probed with the insert from the XLR cDNA clone, pX310. B, Southern comparison of genomic DNA from NZB (lanes *a, e, i*), NZB *xid* (*b, f, j*), CBA/N (*c, g, k*) and CBA/Ca (*d, h*) congenic strains hybridized with XLR probe, pM1. Restriction enzymes were *Bgl*II (*a-d*), *Hinc*II (*e-h*), and *Xba*I (*i-k*). Bands unique to NZB *xid* are indicated by arrows. Using the formulation of Klein¹⁴ and the fact that BXSB *xid*, MRL *xid* and NZB *xid* mice had been bred through 9, 12 and 19 potential recombinations, respectively, at the time of analysis, the probability of linkage to within 10 centimorgans is $1 - (0.43)(0.31) = 87\%$ for the two strains with classic RFLP, and to within 5 centimorgans, 85%, for all three strains.

derived from the normal strain, CBA/Ca. We compared the organization of the XLR locus in genomic DNA from the mutant CBA/N with that from the wild-type CBA/Ca strains, and genomic DNA from a mouse line bearing a distinct X-linked immunodeficiency, DBA/2Ha^{19,20}, with that from its normal parental line, DBA/2J. These comparisons revealed no obvious reorganizations or deletions in the XLR family in either the CBA/N (Fig. 2B, lanes *c, g* and *k*) or DBA/2Ha strains (data not shown). However, many eukaryotic mutations are not obvious deletions or translocations and are not directly detectable by Southern analysis²¹⁻²³. It is conceivable that the complexity of the Southern pattern could obscure such a difference, although the patterns given by many different restriction enzymes have been analysed.

Congenic strains have been bred in which the *xid* mutation, was introduced onto the background of the MRL strain (MRL *xid*²⁴), the BXSB strain (BXSB *xid*²⁵), and the NZB strain (NZB *xid*²⁶); these congenic lines are useful for determining whether a gene probe such as XLR, recognizes DNA linked to the *xid* mutation. The MRL, BXSB and NZB backgrounds are wild type for the *xid* phenotype. The congenic mice are bred by backcrossing male offspring, which have been phenotyped for the *xid* trait, to background females, taking resulting F₁ (+/*xid*) females (the only circumstance in which recombination can occur) for crossing to background males, and typing their male offspring for use in continuing the line. This breeding strategy creates a line deriving almost all of its genetic material from the background strain, and only a small fraction of genetic material, almost exclusively surrounding the locus being introduced (*xid*), from the donor strain (see Fig. 2 legend for calculations)¹⁴. In all cases these mice are statistically expected to derive less than $\frac{1}{8}$ of their X chromosome from the *xid* donor¹⁴. If the XLR probe recognizes RFLPs derived from the *xid* donor, then some (or all) of the gene family must be linked to *xid*, as any unlinked DNA derived from the CBA/N background has essentially been bred out of the line.

Figure 2A shows the Southern blot analysis of male liver DNA prepared from the indicated murine strains, and used in RFLP analysis of the XLR gene family: both MRL *xid* (lane *b*) and BXSB *xid* (lane *f*) congenic mice possess a 5.2-kilobase

(kb) *Pvu*II band of the XLR family characteristic of CBA/N (*xid*) DNA (lane *c*) and unlike the banding pattern of the background strains, MRL (lane *a*) and BXSB (lane *e*). Furthermore, the MRL *xid* mice possess a 9.6-kb *Bgl*II polymorphism (lane *h*), not present in the MRL strain (lane *g*), that is characteristic of *xid* DNA (lane *i*). The BXSB *xid* strain (lane *k*) has inherited two *Bam*HI polymorphisms (at 3.4 and 2.5 kb) from the *xid* background (lane *l*) that are absent from the BXSB (lane *j*). In contrast, BXSB *xid* mice (lane *k*) also have the BXSB (lane *j*) form of a 15-kb *Bam*HI polymorphism, indicating that the BXSB *xid* line has inherited some of its XLR locus from the CBA/N DNAs, and some from the BXSB. If all members of the gene family are tightly clustered, like the MHC genes¹¹ and most gene families, recombination must have occurred between members of the XLR family. If the family is more dispersed, some members will be linked and more distant members unlinked. In either case, we conclude that one or more members of the XLR gene family are closely linked to *xid*.

Interestingly, in comparing multiple restriction digests from the NZB, NZB *xid* and CBA/N DNAs, rather than observing distinct RFLPs, we found bands unique to the NZB *xid* genome that were not present in either of the parental DNAs (Fig. 2B, lanes *b, f* and *j*). The 5.5-kb *Bgl*II, 3.6-kb *Hinc*II and 2.9-kb *Xba*I restriction fragments unique to the NZB *xid* strain are indicated by arrows (Fig. 2B). This observation suggests that during the breeding of the NZB *xid* line, meiotic rearrangement of the XLR locus occurred. The fact that this distinct pattern has been perpetuated for three subsequent generations, and not lost in backcrossing (D.I.C., A.D.S. and M.M.D., unpublished observation), also argues for linkage of XLR to the *xid* phenotype in this congenic strain. Although these findings cannot be unambiguously interpreted, they may represent the result of an inversion or unequal recombination between distinct members of the gene family during the breeding of this strain. In the globin gene family, unequal recombinational events have been associated with gene deletions and reorganizations²⁷.

The multiple members of the XLR gene family may encode functionally distinct molecules and thus might be responsible for more than one of the X-linked deficiencies noted in Table

1. One such possibility is raised by the fact that the human X-linked Wiskott-Aldrich immunodeficiency causes a B-cell defect very similar to that of the *xid* mouse, but in addition leads to abnormal T cells and platelets¹. Consistent with this possibility, XLR genes are expressed in both B and T lymphocytes (see accompanying report⁶). The isolation of the XLR probe described here defines a large number of new X-linked genes, at least some of which are linked to a murine immunodeficiency trait and expressed in lymphocytes. Further,

it offers an important basis for the analysis of X-linked defects in the mouse and, when human probes become available, in the human equivalents.

We thank A. DeFranco for a critical review of the manuscript, T. Waldmann for helpful discussions and K. Redman for assistance in preparing the manuscript. D.I.C. is a Fellow and P.D. a scholar of the Leukemia Society of America. This work has been supported, in part, by a NIH grant to M.M.D. (AI20320), and by NIH grants to P.D. and F.R.

Received 7 September; accepted 10 December 1984.

1. Waldmann, T. A., Strober, W. & Blaese, R. M. in *Clinical Immunology* Vol. 1, 314-375 (W. B. Saunders, Philadelphia, 1980).
2. Scher, I. *Adv. Immun.* **33**, 1-71 (1982).
3. Ohno, S. *A. Rev. Genet.* **3**, 495-524 (1969).
4. Davis, M. M. *et al. UCLA Symp.* **24**, 215-220 (1982).
5. Amsbaugh, D. F. *et al. J. exp. Med.* **136**, 931-949 (1972).
6. Cohen, D. I., Steinberg, A. D., Paul, W. E. & Davis, M. M. *Nature* **314**, 372-374 (1985).
7. Davis, M. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 2194-2198 (1984).
8. Southern, E. M. *J. molec. Biol.* **98**, 503-517 (1975).
9. Shin, H. S., Stavnezer, J., Artzt, K. & Bennett, D. *Cell* **29**, 969-976 (1982).
10. Silver, L. M. *Cell* **29**, 961-968 (1982).
11. Steinmetz, M., Winoto, A., Minard, K. & Hood, L. *Cell* **28**, 489-498 (1982).
12. Goeddel, D. V. *et al. Nature* **290**, 20-26 (1981).
13. Steffen, D. & Weinberg, R. A. *Cell* **15**, 1003-1010 (1978).
14. Klein, J. in *Biology of the Mouse Histocompatibility Complex*, 16-39 (Springer, New York, 1975).

15. Owerbach, D. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 3123-3127 (1981).
16. Paul, W. E. *et al. in Cells of Immunoglobulin Synthesis*, 383-396 (Academic, New York, 1979).
17. Mosier, D. E. *et al. Immun. Rev.* **37**, 89-104 (1977).
18. Ahmed, A. *et al. J. exp. Med.* **145**, 101-110 (1977).
19. Takatsu, K., Tominaga, A. & Hamaoka, T. *J. Immun.* **124**, 2414-2422 (1980).
20. Takatsu, K. *et al. Nature* **292**, 360-362 (1981).
21. Orkin, J. H. *et al. Nature* **296**, 627-631 (1982).
22. Giannelli, F. *et al. Nature* **303**, 181-182 (1983).
23. Wiginton, D. A. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 7481-7485 (1983).
24. Steinberg, E. B. *et al. J. Immun.* **131**, 2789-2795 (1983).
25. Smith, H. R., Chused, T. M. & Steinberg, A. D. *J. Immun.* **131**, 1257-1262 (1983).
26. Steinberg, A. D. *et al. Immun. Rev.* **55**, 121-154 (1981).
27. Jones, R. W., Old, J. M., Trent, R. J. & Weatherall, D. J. *Nature* **291**, 39-44 (1981).
28. Maniatis, T., Fritsch, E. F. & Sambrook, J. in *Molecular Cloning*, 447 (Cold Spring Harbor Laboratory, New York, 1982).
29. D'Eustachio, P., Bothwell, A. L. M., Tokoro, T. K., Baltimore, D. & Ruddle, F. H. *J. exp. Med.* **153**, 793-800 (1981).
30. Goff, S., D'Eustachio, P., Ruddle, F. H. & Baltimore, D. *Science* **218**, 1317-1319 (1982).

Expression of an X-linked gene family (XLR) in late-stage B cells and its alteration by the *xid* mutation

David I. Cohen^{††}, Alfred D. Steinberg[‡], William E. Paul[†] & Mark M. Davis^{*†}

^{*} Department of Medical Microbiology, Stanford University School of Medicine, Stanford, California 94305, USA

[†] Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, and [‡] Arthritis and Rheumatism Branch, NIADDK, National Institutes of Health, Bethesda, Maryland 20205, USA

One of the most extensively studied X-linked immunodeficiency disorder is the *xid* mutation of the mouse strain CBA/N (reviewed in ref. 1). This mutation may involve a maturational defect as *xid* animals are unable to raise antibodies to soluble polysaccharide antigens, a function normally attributed to late-stage B cells^{2,3}. Moreover, studies using monoclonal antibodies have defined a B-cell surface antigen (BLA-2 or 14G8) that is expressed on most or all immature B lymphocytes, but not on a subpopulation of mature splenic B lymphocytes^{4,5}; this late-stage, 14G8 antigen-negative splenic B-cell subpopulation is apparently absent from mice bearing the *xid* defect⁵. In the accompanying paper⁶ we describe the isolation of a cDNA clone recognizing a family of genes on the X chromosome, at least some of whose members are closely linked to the *xid* trait. We report here that this gene family, XLR, is transcribed in certain B- and T-cell lineage tumours, but not in macrophage tumours, or liver or kidney cells. We show that it is transcribed principally in late-stage, 14G8-negative B-cell tumours and plasmacytomas, but not in immature B-cell or pre-B-cell tumours. We are able to detect transcription in all of 12 plasmacytomas (secretory B-cell tumours) derived from mice with normal X chromosomes, but not in three plasmacytomas carrying the *xid* mutation. These data, combined with the restriction fragment length polymorphism analysis linking the XLR gene family to the *xid* mutation⁶, suggests that the *xid* defect occurs within a member of this gene family.

We analysed RNA expression from the XLR gene family in several B-cell tumours and other tissues, by Northern analysis and, in some cases, primer extension studies. Expression of XLR occurs in only certain B-cell tumours (Figs 1, 2); it is also transcribed in a wide variety of functional T-cell hybridomas,

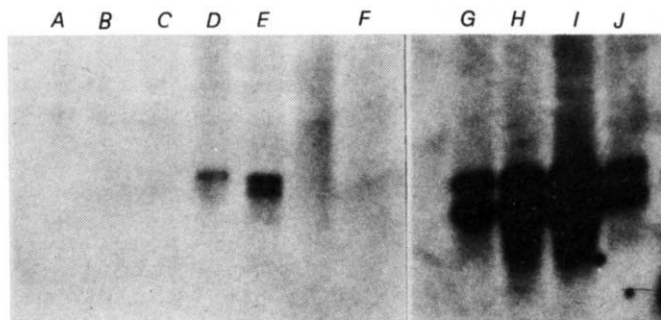


Fig. 1 Northern analysis of 5 µg poly(A)⁺ RNA electrophoresed through 5 mm MeHgOH and 1.5% agarose, as described elsewhere³⁰. Lanes A-F were probed with pX310, a cDNA clone recognizing the XLR gene family; lanes G-J were probed with a β_2 -microglobulin cDNA clone³¹. The RNA samples were from kidney (A, G), liver (B, H), BCL1 (C, I), M315P (non-secretory surface IgA⁺ lymphoma, D), M315J (secretory IgA plasmacytoma, E), and 2043-1 (F, J). In all cases blots were probed with an XLR clone, then with a β_2 -microglobulin clone to ensure that each RNA was intact and hybridizable.

of both helper and suppressor phenotype, and in other T-cell tumours. In contrast, liver cells, kidney cells (Fig. 1A, B) and the macrophage tumour lines J774 (ref. 7) and P338D1 (ref. 8) (data not shown) produced no detectable XLR transcripts. We detected transcription in normal lymphoid tissue by Northern analysis of splenocytes (Fig. 2E) and thymocytes, or by primer extension studies of purified B cells⁹ (data not shown). We conclude that XLR is expressed in some B- and T-cell lymphomas, and in normal lymphocytes, but not in several kinds of non-lymphoid cells; the specificity of this expression during B-cell ontogeny is discussed below.

The level of transcription of XLR genes is ~5-10 copies per cell for XLR-expressing B-cell tumours. This measurement reflects both the frequency of positively hybridizing clones from a plasmacytoma cDNA library (1 clone per 50,000 colonies) and the ratio of XLR to μ (heavy chain) RNA in the presecretory B lymphoma L10A, which is 1/50 as assayed by cytoplasmic RNA titration¹⁰. This level of expression makes it rare¹¹, and it is one of several rare lymphocyte-specific cDNA clones which we have isolated using subtracted cDNA probes^{12,13}. Interestingly, XLR expression seems to be even lower in normal cell populations (Fig. 2, compare lanes B-D with E)¹⁴. Scanning

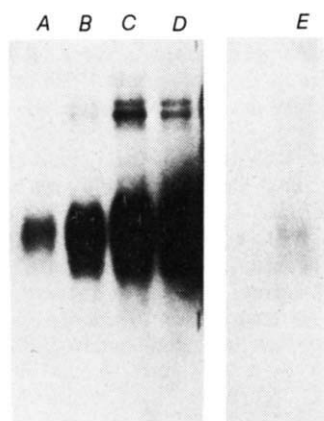


Fig. 2 Northern analysis of total RNA electrophoresed through 1M formaldehyde, 1.5% agarose and blotted onto nitrocellulose, as described elsewhere³². The probe is pM1, a full-length XLR cDNA clone. RNAs in lane A (10 µg) and B–D (20 µg) were from NZB plasmacytomas (2154 (A), 2454 (B), 2880 (C) and 2997 (D) from M. Weigart); E, whole NZB splenocyte RNA (25 µg). The results are from the same blot and probe but lane E (21-day exposure) was exposed seven times longer than lanes A–D (3-day exposure).

densitometry establishes that the most weakly hybridizing NZB plasmacytoma produces a signal 25-fold more intense than NZB splenocytes in equivalent assay conditions; this relationship remains unchanged when polyadenylated RNAs are compared (data not shown).

As XLR expression is observed in only some B-cell tumours, we attempted to determine whether the types of B-cell tumours expressing XLR RNA represent a specific stage of B-cell differentiation. We addressed this question by analysing an extensive panel of B-cell tumours (Table 1), because such tumours have been an effective model for normal B-cell maturation in many systems^{15,16}. The earliest identifiable normal B-cell precursor is the pre-B cell¹⁷. As shown in Table 1, only one of nine Abelson pre-B-cell tumours¹⁶ expresses XLR. Immature B cells, such as those found in neonatal or *xid* mutant mice, possess large amounts of surface IgM¹⁸ and typically express the 14G8 determinant^{4,5}; neither of the two early-stage B-cell tumours assayed here transcribes XLR. More mature B cells express δ (heavy chain)¹⁹ and many do not express 14G8 (refs 5, 7). Furthermore, as a requirement for immunoglobulin secretion, presecretory B cells must activate the gene encoding the J-chain protein because this protein is necessary for assembling the secreted, multimeric forms of IgM and IgA²⁰; therefore, activation of the J-chain gene provides an independent means of assessing B-cell maturity²¹. Of six B-cell lymphomas analysed which possess a mature

surface immunoglobulin phenotype, three are relatively more mature in that they express little or no 14G8, and in that they have activated the J-chain gene as demonstrated by either demethylation (L10A)²¹ or actual transcription (Fig. 3C, lanes e and g). Of this group of six lymphomas, only the three more mature, presecretory B-cell-stage tumours transcribe XLR. The final stage of B-cell differentiation is immunoglobulin secretion and all of 12 secretory-stage tumours (plasmacytomas) transcribe XLR. The pattern of XLR transcription differs between plasmacytomas, which produce two principal transcripts of 1.0 and 1.1 kilobases (kb), and presecretory tumours, which produce one distinguishable transcript of 1.2 kb (Fig. 1D, E). This finding indicates that either multiple members of the family are activated sequentially or differential splicing occurs during the transition from the presecretory to the secretory stage, as occurs with the μ heavy-chain gene²². Even though these stages are probably not absolute, B-cell tumours which transcribe XLR are mature cells at either the presecretory or secretory stage when analysed by multiple criteria (Table 1).

It has been established that a mature subpopulation of splenic B lymphocytes lacking the 14G8 (BLA-2) antigen, and uniquely characterized by a panel of other markers, is absent from mice with the *xid* mutation⁵. Interestingly, the B-cell tumours analysed in the present report, which transcribe XLR, also lack the 14G8 marker. On the basis of this similarity, it is tempting to equate these B-cell tumours expressing XLR with the normal B cells which are absent from *xid* mutant mice. The 'turning-on' of this X-linked gene during the maturation of some B cells coincides with the 'turning-off' of a marker (14G8) which is expressed at an abnormally high frequency in *xid* B cells.

We next asked whether the *xid* mutation directly affects XLR expression. We prepared and analysed mRNA from a pool of three plasmacytomas raised in the NZB *xid* mouse²³, which is an NZB congenic strain into which the *xid* mutation has been introduced²⁴, and compared the results with those obtained from plasmacytomas lacking the *xid* mutation, including NZB plasmacytomas. The 12 plasmacytomas lacking the *xid* mutation included cells using the μ , γ or α immunoglobulin heavy chains and κ or λ light chains, and represented tumours expanded either *in vivo* or *in vitro*. All of these 12 plasmacytomas gave essentially similar patterns and amounts of XLR transcription whereas the pool of NZB *xid* plasmacytomas showed no detectable XLR expression (Fig. 3A, lane h). The most relevant comparison is with the four NZB lines (Fig. 2A–D). Allowing for the slightly greater amount of RNA loaded for the NZB *xid* pool, and the sensitivity of these Northern blots (see above), we estimate that XLR transcription must be decreased at least 30-fold in the NZB *xid* plasmacytomas, to less than 1 copy per cell; comparative titrations of plasmacytoma RNAs in primer extension experiments support this conclusion (data not shown). Figure 3C, lane j shows that the same NZB *xid* plasmacytoma RNA contains readily detectable J-chain mRNA, establishing

Table 1 XLR expression in B-cell tumours

Stage	XLR (no. positive/ no. tested)	Phenotype			
		Immunoglobulin	14G8 staining	J chain	Tumour (XLR expression + or -)
(1) Pre-B	1/9	Cytoplasmic μ	+	-	2043-1, 38B7, ABL5 1, 8, 19, 109, 122, 140 [all (-)] ABL5 5 (+)
(2) Early B	0/2	Surface IgM	+	-	BCL1, W231
(3) Intermediate B	0/3	Surface IgM, IgD	+	-	K46, X16C, BAL17
(4) Presecretory B	3/3	Surface immunoglobulin IgD	-	DM or +	L10A*, 315P, 2PK3
(5) Plasmacytoma	12/12	Secretory immunoglobulin	-	+	V17C, HM14, CPC1, TEPC 1197, CBB22PC3, CBPC112, N2BPC 2154, 2454, 2880, 2993, M315J, M104e

DM, demethylated, not expressed²¹. Data were taken from refs 15, 16, 21, 26–29 and the present study.

We tested all tumours in categories (2), (3) and (4) for 14G8 staining and J expression, but only representative tumours from categories (1) and (5).

* Ref. 21.

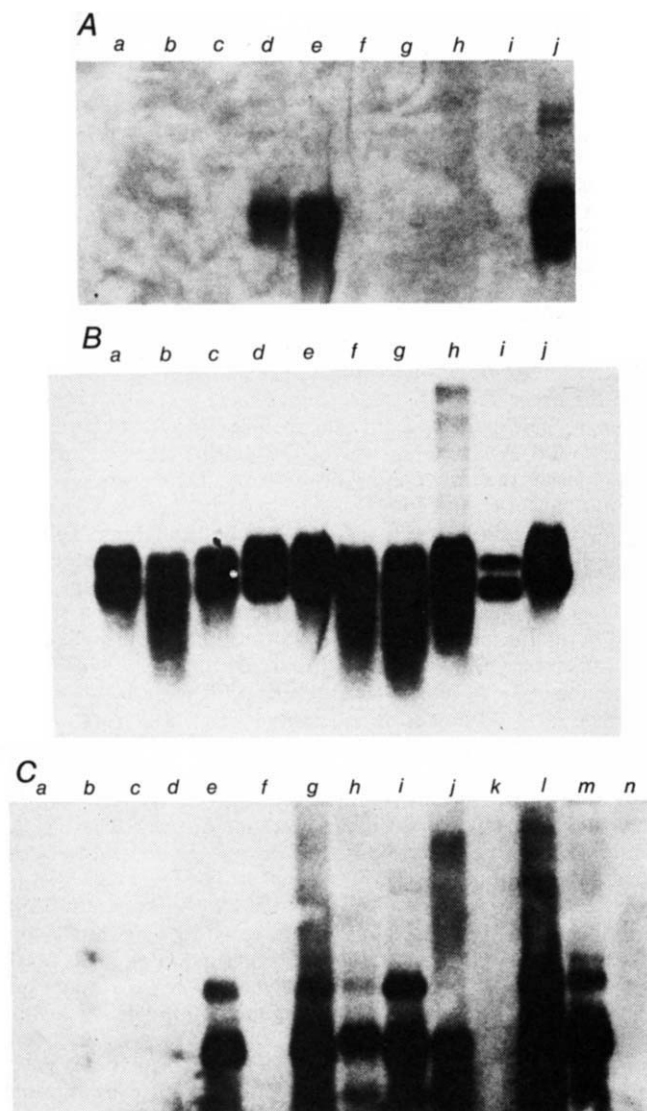


Fig. 3 Northern analysis, as in Fig. 2, using 30 μ g of total NZB *xid* plasmacytoma, or 5 μ g of poly(A)⁺ RNA from the other tumours. **A**, Probed with pM1; **B**, the same blot probed with β_2 -microglobulin. The RNAs in each lane are: *a*, WEH1 231 (W231); *b*, W231 (different batch); *c*, lipopolysaccharide (LPS)-stimulated W231; *d*, 2PK3; *e*, L10A; *f*, X16C; *g*, K46; *h*, NZB *xid* plasmacytoma; *i*, BAL 17; and *j*, M104e. **C**, RNA probed with a J-chain cDNA clone¹⁵; *a*, W231; *b*, BAL17; *c*, X16C; *d*, K46; *e*, 2PK3; *f*, L10A; *g*, M315P; *h*, M104e; *i*, M315J; *j*, NZB *xid* plasmacytoma; *k*, T-cell RNA from EL4; *l*, V17C; *m*, HM14; and *n*, LPS-stimulated W231. The NZB *xid* plasmacytoma RNA was extracted from a pool of equal numbers of cells from three independently derived plasmacytomas, in order to have sufficient material for complete analysis. One principal type of antibody produced by these cells is IgA/ κ (A.D.S., unpublished data).

that these cells are at the correct stage to transcribe XLR (Table 1), and that the mRNA analysed is intact. We conclude that the *xid* mutation alters the normal expression of XLR.

The experiments described here establish that XLR transcription is characteristic of late-stage B-lymphocyte maturation and that this expression is altered in cells bearing the *xid* mutation. These data indicate that the *xid* mutation disrupts XLR gene expression and, coupled with the linkage data described in the accompanying paper⁶, support the notion that the *xid* mutation occurs within a member of the XLR gene family. The mutation may act either directly or indirectly to account for the pattern of expression observed here. In the direct model, the NZB *xid* plasmacytoma cells would normally express XLR but cannot, owing to the *xid* defect. Alternatively, these plasma cells may represent an XLR-nonexpressing class of B cells that are rare

in normal animals but common in those carrying the *xid* defect; this rare class would predominate if the XLR-requiring plasma cell fails to mature in the *xid* animals. The latter alternative is more consistent with a dual-pathway model of (late-stage) B-cell maturation^{1,5,25}.

We thank Dr M. Koshland for the J-chain cDNA clone, Jc-3 used in our study; Dr J. Parnes for the β_2 -microglobulin cDNA clone; Dr A. DeFranco for RNA and for critical review of the manuscript; Drs R. Lynch for the M315P and M315J lines, J. F. Mushinski for ABL and plasma cell RNAs, J. Kung and J. Kemp for the cell-surface staining in Table 1; and K. Redman for assistance in the preparation of the manuscript. D.I.C. is a Fellow of the Leukemia Society of America. This work has been supported, in part, by a NIH grant to M.M.D. (AI20320).

Received 7 September; accepted 12 December 1984.

1. Paul, W. E. *et al.* in *Cells of Immunoglobulin Synthesis*, 383-396 (Academic, New York, 1979).
2. Ahmed, A. *et al.* *J. exp. Med.* **145**, 101-110 (1977).
3. Mosier, D. E. *et al.* *Immun. Rev.* **37**, 89-104 (1977).
4. Kung, J. T. *et al.* *J. Immun.* **128**, 2049-2056 (1982).
5. Hardy, R. R., Hayakawa, K., Parks, D. R., Herzenberg, L. A. & Herzenberg, L. A. *J. exp. Med.* **159**, 1169-1188 (1984).
6. Cohen, D. I. *et al.* *Nature* **314**, 369-372 (1985).
7. Lachman, L. B., Hacker, M. P., Blyden, G. T. & Handschumacher, R. E. *Cell. Immun.* **34**, 416-418 (1977).
8. Mizel, S. B., Rosenstreich, D. L. & Oppenheim, J. J. *Cell. Immun.* **40**, 230-241 (1978).
9. Howard, M. *et al.* *J. exp. Med.* **155**, 914-923 (1982).
10. Kemp, D. J., Harris, A. W., Cory, S. & Adams, J. M. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2876-2880 (1980).
11. Lewin, B. in *Gene Expression* Vol. 2, 2nd edn, 713-719 (Wiley, New York, 1980).
12. Hedrick, S. M., Cohen, D. I., Nielsen, E. A. & Davis, M. M. *Nature* **308**, 149-153 (1984).
13. Davis, M. M., Chien, Y. H., Gascoigne, N. R. J. & Hedrick, S. M. *Immun. Rev.* **81**, 235-258 (1984).
14. Payne, G. S., Bishop, J. M. & Varmus, H. E. *Nature* **295**, 209-217 (1982).
15. Mather, E. L., Alt, F. W., Bothwell, A. L. M., Baltimore, D. & Koshland, M. E. *Cell* **23**, 369-389 (1981).
16. Siden, E. J., Baltimore, D., Clark, D. & Rosenberg, N. *Cell* **16**, 389-396 (1979).
17. Levitt, D. & Cooper, M. D. *Cell* **19**, 617-625 (1980).
18. Scher, I., Sharrow, S. O., Wistar, R. Jr, Asofsky, R. & Paul, W. E. *J. exp. Med.* **144**, 494-506 (1976).
19. Kearney, J. F. *et al.* *J. exp. Med.* **146**, 297-301 (1977).
20. Koshland, M. E. *Adv. Immun.* **20**, 41-69 (1975).
21. Yagi, M. & Koshland, M. E. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4907-4911 (1981).
22. Rogers, J. *et al.* *Cell* **20**, 303-312 (1980).
23. Steinberg, B. J. *et al.* *J. clin. Invest.* **70**, 587-597 (1982).
24. Klein, J. in *Biology of the Mouse Histocompatibility Complex*, 16-39 (Springer, New York, 1975).
25. Perlmutter, R. M. *et al.* *J. exp. Med.* **149**, 993-998 (1979).
26. Kim, K. J. *et al.* *J. Immun.* **122**, 549-554 (1979).
27. Lanier, L. L., Warner, N. L., Ledbetter, J. A. & Herzenberg, L. A. *J. Immun.* **127**, 1691-1697 (1981).
28. Slavin, S. & Strober, S. *Nature* **272**, 624-626 (1978).
29. Gebel, H. M., Autry, J. R., Rohrer, J. W. & Lynch, R. G. *J. natn. Cancer Inst.* **62**, 201-212 (1979).
30. Alwine, J. C., Kemp, D. J. & Stark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5350-5354 (1977).
31. Parnes, J. R. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **78**, 2253-2257 (1981).
32. Seed, B. in *Genetic Engineering* Vol. 4 (eds Setlow, J. K. & Hollaender, A.) 91-102 (Academic, New York, 1982).

An N-terminal peptide from p60^{src} can direct myristylation and plasma membrane localization when fused to heterologous proteins

David Pellman*, Ellen A. Garber, Frederick R. Cross & Hidesaburo Hanafusa

The Rockefeller University, 1230 York Ave, New York, New York 10021, USA

The *src* gene product, p60^{src}, of Rous sarcoma virus (RSV) is a tyrosine-specific protein kinase which is associated with the plasma membrane of infected cells (for review, see ref. 1). Myristic acid is bound in an amide linkage to glycine 2 of p60^{src} (refs 2-6). Of the N-terminal 30 kilodaltons of p60^{src}, only amino acids 1-14 are required for myristylation, and myristylation of p60^{src} may be required for its membrane association, and for cell transformation^{4,7}. To test the hypothesis that the first 14 amino acids of p60^{src} contain a recognition sequence for myristylation, we have fused the DNA sequence coding for these amino acids to either the *fps*

* Present address: Division of the Biological Sciences and the Pritzker School of Medicine, University of Chicago, Chicago, Illinois 60637, USA.

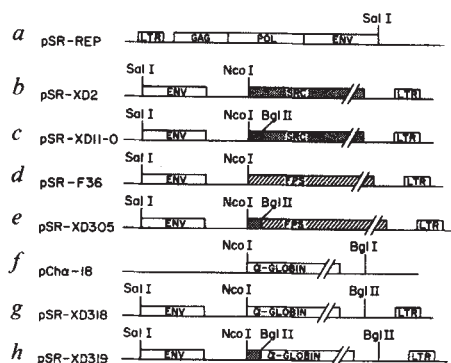


Fig. 1 Structures of the coding sequences of the plasmids used. The shaded boxes indicate portions of the *src* gene; hatched boxes, portions of the *fps* gene; open boxes, chimpanzee α -globin gene. All methods for recombinant DNA manipulations have been described previously^{4,26}. All constructions were verified by restriction mapping, and by DNA sequence analysis in the case of pSR-XD305 and pSR-XD319. *a*, pSR-REP contains most of the genes required for replication of RSV (*gag*, *pol* and *env*), and was used in the transfection protocol to produce infectious virus containing the replacements for the *src* gene shown in *b*–*g*, as described elsewhere²⁶. *b*, pSR-XD2 contains the wild-type RSV *src* gene²⁶. *c*, pSR-XD11-0 is a pSR-XD2 derivative with a *Bgl*III linker inserted into the DNA coding for arginine 15 of p60^{src} (ref. 26). pSR-XD11-4 has a similar structure, except that two more base pairs are inserted into the DNA at the 5' side of the linker²⁶. *d*, pSR-F36 is similar to pSR-XD2, except that the *src* gene is replaced by a transforming fragment of the FSV *fps* gene. The construction and characterization of this clone have been described⁹. *e*, pSR-XD305 encodes a fusion protein between amino acids 1–14 of p60^{src} and amino acids 2–833 of the F36 transforming protein. This clone was constructed by first partially digesting pSR-F36 with *Nco*I, and with *S*₁ nuclease, followed by *Bgl*III linker (CAGATCTG) insertion. One clone, pF23, had a linker inserted at the remnant of the *Nco*I site which spans the initiation codon of the transforming protein of F36 (ref. 9), with *S*₁ digestion of only the protruding 5' end remaining after *Nco*I digestion. This clone was recombined, at its *Bgl*III site, with pSR-XD11-0. *f*, pCh α -18 is a complete cDNA clone of a chimpanzee α -globin gene¹⁰. *g*, pSR-XD318, a derivative of pSR-XD2 with the pCh α -18 coding sequence substituted for *src*. pCh α -18 was cut with *Bgl*I (which cuts 3' to the termination codon) followed by *S*₁ digestion and *Bgl*III linker insertion. This DNA was digested with *Bgl*III and *Nco*I. *Nco*I cuts at the initiation codon for α -globin. pSR-XDR111, a pSR-XD2 derivative with a *Bgl*III linker inserted at the *Rsa*I site 172 nucleotides 3' to the termination codon of *src* (F.R.C., unpublished data), was digested with *Bgl*II and partially with *Nco*I. The fragment that was digested with *Nco*I only at the *src* initiation codon was ligated to the globin DNA insert to produce pSR-XD318. *h*, pSR-XD319 is a derivative of pSR-XD2 which encodes a fusion between the first 14 amino acids of p60^{src} and all of α -globin. pCh α -18 was cut with *Bgl*II and *S*₁, then with *Nco*I. The *Nco*I site was gap-filled, and *Bgl*III linkers ligated to this insert DNA. pSR-XD321, a pSR-XD2 derivative in which all of *src* except for DNA encoding amino acids 1–14 is deleted (J. Samarut and F.R.C., unpublished), was constructed by recombining pSR-XD11-4 (see description of pSR-XD11-0 above) with pSR-XDR23, a pSR-XD2 derivative with a *Bgl*III linker inserted at an *Rsa*I site 105 nucleotides 3' to the *src* termination codon (F.R.C., unpublished). After cutting of pSR-XD321 with *Bgl*II, followed by digestion with alkaline phosphatase, the *Bgl*III-ended α -globin insert was ligated into the plasmid.

gene of the F36 derivative of Fujinami sarcoma virus (FSV)^{8,9}, or to the chimpanzee α -globin gene¹⁰. We report here that although the fusion proteins were myristylated, the parental proteins were not, and unlike the non-myristylated F36 p91^{fps} which was not bound to the plasma membrane, the myristylated fusion protein was bound, like p60^{src}. We conclude that the first 14 amino acids of p60^{src} contain a sequence which is sufficient for myristylation, and which may direct proteins to the plasma membrane.

The structures of the genes inserted into the RSV genome instead of the *src* gene are shown in Fig. 1. Figure 2 shows the sequences of regions of interest.

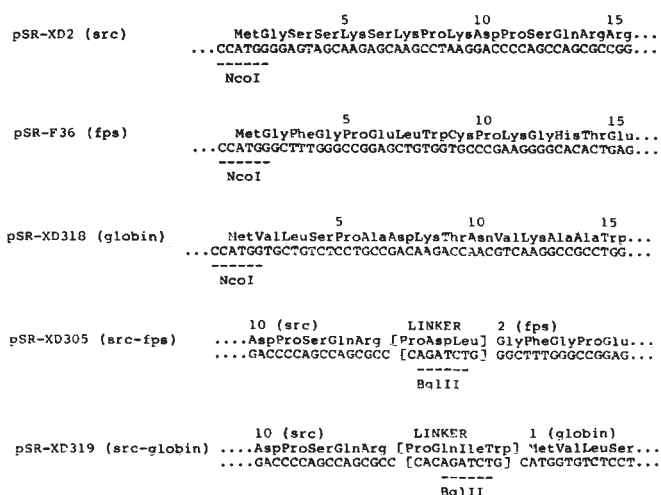


Fig. 2 Sequences of the N-terminal portions of the coding sequences in pSR-XD2, pSR-F36, pSR-XD318, pSR-XD305 and pSR-XD319. The pSR-XD2, pSR-F36 and pSR-XD318 sequences are from refs 27, 8 and 10, respectively. DNA derived from *Bgl*III linkers and amino acids encoded by this DNA are shown in brackets. The *src* DNA in pSR-F36 is derived from pSR-XD11-0, and that in pSR-XD319 from pSR-XD11-4 (ref. 26).

The fragment of the *fps* gene expressed following transfection with the pSR-F36 plasmid (nucleotides 1,472–4,162 of the FSV *fps* gene) lacks the *gag* portion, and encodes an 833-amino acid *fps* protein (amino acids 349–1,182 of the FSV *fps* protein), called p91^{fps}. The plasmid pSR-XD305 (Figs 1, 2) contains a recombinant *src*–*fps* gene which encodes a protein consisting of the first 14 amino acids of p60^{src}, followed by Pro-Asp-Leu, then amino acids 2–833 of the F36 *fps* protein⁹. The viruses recovered following transfection with these plasmids are called F36 (ref. 9) and NY305, respectively.

A protein of relative molecular mass (*M*_r) 93,000 (93K) is specifically immunoprecipitated from extracts of NY305-infected cells by anti-*fps* antiserum (Fig. 3A). Proteolytic mapping (data not shown) confirms that this protein is the product of the NY305 *src*–*fps* fusion gene, p93^{src-fps}. The F36 p91^{fps} is not detectably labelled with ³H-myristic acid, even after long exposure, whereas both p60^{src} and p93^{src-fps} label well (Fig. 3A).

We have compared the subcellular localization of p91^{fps} and p93^{src-fps} by cell fractionation and measurement of immune complex tyrosine kinase activity, as described previously^{4,9}. As with the FSV transforming protein p140^{gag-fps} (refs 11–13), the proportion of p91^{fps} found in the crude membrane pellet (P100) was salt-dependent (Fig. 4A). In contrast, 95% of the p93^{src-fps} was found in the crude membrane pellet at both salt concentrations tested. The P100 fractions were floated in discontinuous sucrose gradients. Unlike p60^{src}, which is tightly and specifically associated with the plasma membrane¹, the p91^{fps} kinase activity was not enriched in any membrane fraction. The p93^{src-fps} kinase activity was highly enriched in plasma membranes (Fig. 4B). Therefore, the addition of the first 14 amino acids of p60^{src} to p91^{fps} made the subcellular fractionation pattern of the fusion protein indistinguishable from that of p60^{src} (refs 1, 4, 14).

Both F36 and NY305 induce focus formation efficiently, and NY305 induces the same distinctive fusiform morphology as reported for F36, in contrast to the round cell morphology induced by RSV⁹ (data not shown). As the RSV p60^{src} and NY305 p93^{src-fps} proteins appear to have a similar subcellular location, transformed cell morphology may reflect the affinity of these enzymes for substrates, rather than the subcellular location of the tyrosine kinase. Like F36, NY305 induces colony formation in soft agar, and both viruses are tumorigenic (data not shown). Thus, the alteration in subcellular location of p91^{fps} by the addition of the first 14 amino acids of p60^{src} did not alter

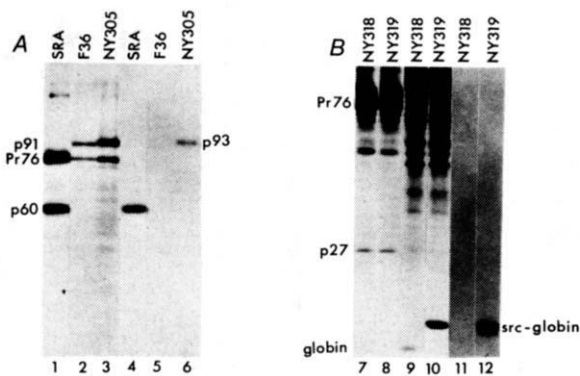


Fig. 3 Myristylation of fusion proteins containing the N-terminal 14 amino acids of $p60^{src}$. Infected chick embryo fibroblasts (CEF) were labelled for 4 h with 3H -leucine or 3H -myristic acid, and cell lysates were immunoprecipitated with saturating amounts of anti-serum as described previously⁴. **A**, Infected cells were labelled with 3H -leucine (lanes 1-3) or 3H -myristic acid (lanes 4-6), and immunoprecipitated proteins were separated by electrophoresis on a 9% SDS-polyacrylamide gel. Proteins from cells infected with the Schmidt-Ruppin subgroup A strain of RSV (SRA; lanes 1, 4) were immunoprecipitated by tumour-bearing rabbit (TBR) serum⁴. Proteins from F36- (lanes 2, 5) or NY305-infected cells (lanes 3, 6) were immunoprecipitated by FSV-specific regressing-tumour antiserum (anti-FST; refs 9, 28). Exposure of the fluorographed gel for 2 days yielded lanes 1-3; exposure for 12 days lanes 4-6. **B**, Infected cells were labelled with 3H -leucine for 0.5 h (lanes 7-10) or with 3H -myristic acid for 2 h (lanes 11, 12), and immunoprecipitated proteins were analysed by electrophoresis on a 12.5% SDS-polyacrylamide gel. Proteins were immunoprecipitated with TBR serum (lanes 7, 8) or with rabbit anti-human haemoglobin serum (Cappel Laboratories) (lanes 9-12). The fluorographed gel was exposed for 12 (lanes 7-10) or 16 (lanes 11, 12) days.

biological activity; this is in contrast to the requirement for myristylation and membrane association of $p60^{src}$ for cell transformation^{4,7}, and demonstrates a biological difference between $p60^{src}$ and $p91^{fps}$. Although recent reports suggest that FSV $p140^{gag-fps}$ is loosely associated with the plasma membrane^{12,13}, we have not observed this for $p91^{fps}$ in our conditions for cell fractionation (Fig. 4B).

As $p60^{src}$ and $p91^{fps}$ are partially homologous⁸, we wanted to test the effect of the first 14 amino acids of $p60^{src}$ on an unrelated protein. We constructed RSV genomes in which the *src* gene is replaced by the gene for chimpanzee α -globin¹⁰, or by a fusion of the sequence coding for the first 14 amino acids of $p60^{src}$ and the α -globin gene (the structures of these recombinants are shown in Figs 1, 2). Cells transfected with these DNAs were labelled with 3H -leucine or 3H -myristic acid. Figure 3B shows the proteins immunoprecipitated from these cells with anti- α -globin antiserum. Cells transfected with pSR-XD318, the plasmid encoding α -globin, produce a 15 K protein, approximately the expected size¹⁰. Cells transfected with pSR-XD319, the plasmid encoding the *src*-globin fusion protein, produce a 17 K protein. Both of these bands are specific to the DNA used for transfection, and are not seen in immunoprecipitates with normal serum, therefore they probably represent α -globin and the *src*-globin fusion protein, respectively. Only the *src*-globin fusion protein is labelled with 3H -myristic acid.

Peptide sequences that direct post-translational modifications have been described elsewhere¹⁵. It is probable that the first 14 amino acids of $p60^{src}$ contain a signal for N-myristylation. However, there is no obvious sequence homology between the N-terminal portions of other myristylated proteins¹⁶⁻¹⁹ and that of $p60^{src}$.

The α -globin protein, and to a lesser extent the *src*-globin fusion protein, are quite unstable in chicken embryo fibroblasts (data not shown), and are therefore difficult to label efficiently

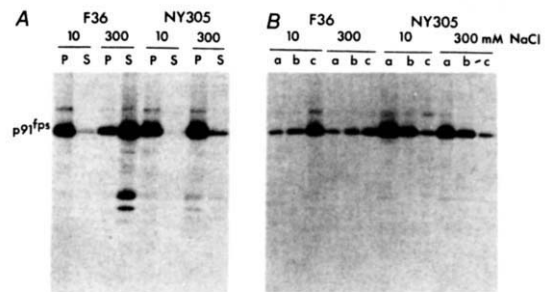


Fig. 4 Salt-independent plasma membrane association of a fusion protein containing the N-terminal 14 amino acids of $p60^{src}$. **A**, Infected cells labelled for 4 h with 3H -leucine were fractionated into crude membrane (P) and cytosolic (S) fractions as described previously⁴, except that the salt concentration of the postnuclear supernatant was adjusted to 10 mM or 300 mM as indicated. Kinase activity was assayed in immune complexes formed by immunoprecipitation with anti-FST serum as described previously^{9,28}. Immunoprecipitates were analysed on a 9% polyacrylamide gel. Autophosphorylated bands were excised from the gel shown in Fig. 3A, and ^{32}P radioactivity quantitated by Cerenkov counting. The percentage radioactivity found in the P100, assuming 100% recovery, was as follows: for F36 $p91^{fps}$, 94% and 15% at 10 and 300 mM NaCl, respectively; for NY305 $p93^{src-fps}$, 96% and 93%, respectively. **B**, Crude membrane fractions prepared in **A** were fractionated on a discontinuous sucrose gradient as described previously⁴, except that the gradient contained fewer steps: the P100 fraction was resuspended in 40% sucrose, overlaid with 50% sucrose, and overlaid with 35% and 20% sucrose in buffer. Lanes a-c represent membrane fractions collected from the gradient interfaces: a, 20/35% sucrose interface, which is enriched for plasma membranes; b, 35/40% sucrose interface; c, 40/50% sucrose interface, which is enriched for rough endoplasmic reticulum, Golgi and mitochondrial membranes¹⁴. Kinase activity was assayed in immune complexes formed by immunoprecipitation with anti-FST serum and analysed by electrophoresis on a 9% SDS-polyacrylamide gel. The radioactivity in the autophosphorylated bands was quantitated, and the specific activity of kinase activity in each fraction quantitated with reference to the total protein content, as described elsewhere⁴. $p91^{fps}$ distributed essentially in parallel with the total protein of the fractions. $p93^{src-fps}$ showed an approximately 100-fold enrichment in the 20/35% fraction relative to that in the 40/50% fraction.

with 3H -leucine. We have been unable, therefore, to determine the subcellular localization of the α -globin protein. However, the *src*-globin fusion protein is found primarily in a crude membrane pellet after extraction of cells in isotonic salt (data not shown), which is consistent with the $p60^{src}$ N-terminus having a similar effect on α -globin as it has on $p91^{fps}$ with respect to subcellular localization.

The Harvey sarcoma virus transforming protein, $p21^{ras}$, is covalently modified with palmitic acid², a modification which requires a short C-terminal sequence and correlates with p21 membrane association²⁰. Short sequences which target proteins to their appropriate subcellular locations have been described for secreted proteins, mitochondrial proteins, integral membrane proteins, and nuclear proteins²¹⁻²⁵. The results presented here show that the myristylated N-terminal 14 amino acids of $p60^{src}$ contain a sequence which is sufficient for myristylation, and which may direct $p60^{src}$ and $p93^{src-fps}$ (and possibly the *src*-globin fusion protein) to the plasma membrane.

We thank D. Foster for the gift of pSR-F36 plasmid and anti-FST antiserum; Stephen A. Lieberhaber for the gift of pCH α -18 α -globin cDNA; Janice E. Buss for communicating results before publication; and S. Sugano, D. Foster and A. Dutta for comments on the manuscript. This work was supported by USPHS grant CA14935 from the NCI, and by grant MV128B from the American Cancer Society. E.A.G. was supported by a Merck fellowship. F.R.C. was supported by NI training grant AI07233.

Received 30 October; accepted 18 December 1984.

1. Krueger, J. G., Garber, E. A. & Goldberg, A. R. *Curr. Topics Microbiol. Immun.* **107**, 51-124 (1983).
2. Sefton, B. M., Trowbridge, I. S., Cooper, J. A. & Scolnick, E. M. *Cell* **31**, 465-474 (1982).
3. Garber, E. A., Krueger, J. G., Hanafusa, H. & Goldberg, A. R. *Nature* **302**, 161-163 (1983).
4. Cross, F. R., Garber, E. A., Pellman, D. & Hanafusa, H. *Molec. cell. Biol.* **4**, 1834-1842 (1984).
5. Buss, J. E. & Sefton, B. M. *J. Virol.* **53**, 7-12 (1984).
6. Schultz, A. M., Henderson, L. E., Oroszlan, S., Garber, E. A. & Hanafusa, H. *Science* **227**, 427-429 (1984).
7. Pellman, D., Garber, E. A., Cross, F. R. & Hanafusa, H. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
8. Shibuya, M. & Hanafusa, H. *Cell* **30**, 787-795 (1982).
9. Foster, D. A. & Hanafusa, H. *J. Virol.* **48**, 744-751 (1983).
10. Liebhafner, S. A. & Begley, K. A. *Nucleic Acids Res.* **11**, 8915-8929 (1983).
11. Feldman, R. A., Wang, E. & Hanafusa, H. *J. Virol.* **45**, 782-791 (1983).
12. Woolford, J. & Beemon, K. *Virology* **135**, 168-180 (1984).
13. Moss, P. *et al. J. Virol.* **52**, 557-565 (1984).
14. Courtneidge, S. A., Levinson, A. D. & Bishop, J. M. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3783-3787 (1980).
15. Wold, F. A. *Rev. Biochem.* **50**, 783-814 (1981).
16. Carr, S. A., Biemann, K., Shoji, S., Parmelee, D. C. & Titani, K. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6128-6131 (1982).
17. Aitken, A. *et al. FEBS Lett.* **150**, 314-318 (1982).
18. Henderson, L. E., Krutzsch, H. C. & Oroszlan, S. *Proc. natn. Acad. Sci. U.S.A.* **80**, 339-343 (1983).
19. Schultz, A. M. & Oroszlan, S. *Virology* **133**, 431-437 (1984).
20. Willumsen, B. M., Christensen, A., Hubbert, N. L., Papageorge, A. G. & Lowy, D. R. *Nature* **310**, 583-586 (1984).
21. Kreil, G. A. *Rev. Biochem.* **50**, 317-348 (1981).
22. Schatz, G. & Butow, R. A. *Cell* **32**, 316-318 (1983).
23. Hall, M. N., Hereford, L. & Herskowitz, I. *Cell* **36**, 1057-1065 (1984).
24. Kalderon, D., Richardson, W. D., Markham, A. F. & Smith, A. E. *Nature* **311**, 33-38 (1984).
25. Walter, P., Gilmore, R. & Blobel, G. *Cell* **38**, 5-8 (1984).
26. Cross, F. R. & Hanafusa, H. *Cell* **34**, 597-607 (1983).
27. Takeya, T. & Hanafusa, H. *J. Virol.* **44**, 12-18 (1982).
28. Mathey-Prevot, B., Hanafusa, H. & Kawai, S. *Cell* **28**, 897-906 (1982).

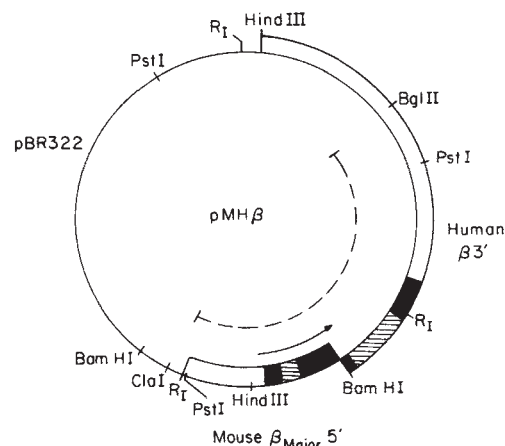


Fig. 1 Restriction map of plasmid pMH β , containing a hybrid mouse/human β -globin gene. This plasmid was provided by P. Mellon and T. Maniatis, and its construction is described in ref. 4. The plasmid contains the 5' portion of the mouse β -major globin gene and the 3' portion of the human β -globin gene, fused at a conserved *Bam*HI site in the second exon of both genes. The clone also includes 1.2 kb of 5'-flanking mouse DNA and 3.8 kb of 3'-flanking human DNA. Solid bars represent exons, hatched bars represent introns, and open bars flanking mouse or human DNA. The broken line indicates the 5.0-kb *Cla*I-*Bgl*II fragment used for microinjection in some experiments.

Specific expression of a foreign β -globin gene in erythroid cells of transgenic mice

Kiran Chada, Jeanne Magram, Kathryn Raphael*, Glenn Radice, Elizabeth Lacy* & Frank Costantini

Department of Human Genetics and Development, College of Physicians and Surgeons, Columbia University, 701 W. 168th Street, New York, New York 10032, USA

The globin gene family represents an attractive system for the study of gene regulation during mammalian development, as its expression is subject to both tissue-specific and temporal regulation. While many aspects of globin gene structure and expression have been described extensively¹, relatively little is known about the *cis*-acting DNA sequences involved in the developmental regulation of globin gene expression. To begin to experimentally define these regulatory sequences, we have taken the approach of introducing cloned globin genes into the mouse germ line and examining their expression in the resulting transgenic animals^{2,3}. Here we describe a series of transgenic mice carrying a hybrid mouse/human adult β -globin gene, several of which express the gene exclusively or predominantly in erythroid tissues. These studies demonstrate that regulatory sequences closely linked to the β -globin gene are sufficient to specify a correct pattern of tissue-specific expression in a developing mouse, when the gene is integrated at a subset of foreign chromosomal positions.

Figure 1 shows a restriction map of the hybrid β -globin gene⁴ used in these experiments. A portion of the mouse β -major globin gene and 5'-flanking DNA was included to ensure that any regulatory sequences within this region would be compatible with regulatory factors in mouse erythroid cells, and to thus circumvent possible species-specific differences in globin gene regulation. The 3' end of the gene was derived from the human β -globin gene, allowing the hybrid gene and its mRNA to be distinguished from the endogenous β -globin genes and their

mRNAs. Mouse zygotes were microinjected^{2,5-7} with either the intact circular plasmid pMH β , or a linear 5.0-kilobase (kb) *Cla*I-*Bgl*II fragment containing the entire gene and 5'-flanking mouse DNA segment, 1.9 kb of 3' flanking human DNA, and only 350 base pairs (bp) of plasmid DNA (Fig. 1). Ten transgenic mice were produced, three carrying the entire plasmid and seven carrying the *Cla*I-*Bgl*II fragment (Table 1). When mated with normal mice, each of these transgenic mice transmitted the foreign DNA to a fraction of its progeny, and 10 transgenic lines were established. Southern blot analysis (data not shown) revealed that each line carries one or more copies of a 3.4-kb *Pst*I fragment including the β -globin gene, nearly all of the 5'-flanking mouse DNA and 0.5 kb of the 3'-flanking human DNA (Fig. 1, Table 1).

To determine the pattern of expression of the foreign β -globin gene in each transgenic line, we performed a quantitative *S*₁ nuclease protection assay^{8,9} on RNA samples isolated from erythroid and non-erythroid mouse tissues. Mice in four of the lines (46, 49, 75A and 77) were found to express the gene predominantly or exclusively in erythroid tissues; see Fig. 2a. The hybridization probe used here derives from the 3' end of the human β -globin gene, and a 212-nucleotide fragment is protected from *S*₁ nuclease digestion by either correctly terminated mRNA encoded by the hybrid β -globin gene, or by human β -globin mRNA⁴. For purposes of comparison, only the 212-nucleotide band obtained with each RNA sample is shown (this was the only reproducible band observed). It can be seen that the highest levels of mRNA derived from the hybrid β -globin gene are found in bone marrow and blood cells.

To confirm that the hybrid β -globin mRNA detected in total blood and bone marrow RNA preparations represented expression in erythroid cells, we analysed RNAs from the tissues of several mice that had been injected with phenylhydrazine, a treatment which causes the spleen to become a major site of erythropoiesis¹⁰. As demonstrated in Fig. 2b for mice in lines 77 and 46, the level of hybrid β -globin mRNA in the spleen after phenylhydrazine treatment was equivalent to that in the bone marrow, while in untreated mice (for example, Fig. 2a) the level in spleen was much lower.

In some animals, relatively low levels of hybrid β -globin mRNA were detected in RNAs from certain non-erythroid tissues, particularly heart, lung and kidney (Fig. 2a, b). We

* Present addresses: CSIRO, Division of Animal Production, Ian Clunies Ross Animal Research Laboratory, Great Western Highway, Prospect, New South Wales, Australia (K.R.); Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021, USA (E.L.).

suspected that much of this mRNA might have derived from blood cells contaminating these tissues rather than from actual transcription of the foreign β -globin gene in non-erythroid cells; this was confirmed by measuring the amount of endogenous β -globin mRNA contaminating different non-erythroid tissues. In general, those non-erythroid tissues containing the highest levels of hybrid β -globin mRNA also contained the highest levels of endogenous mouse β -globin mRNA (an example is shown in Fig. 2c). There were a few non-erythroid tissues (testes of a line 49 mouse and heart and kidney of line 75A mice; Fig. 2a) in which hybrid β -globin mRNA appeared to result from inappropriate expression of the foreign globin gene at a very low level (less than one mRNA molecule per cell).

The steady-state level of hybrid β -globin mRNA in each mouse RNA sample was estimated by comparing the observed hybridization signals with those obtained using known amounts of human β -globin mRNA (Fig. 2, Table 1). The mRNA levels in erythroid tissues were highest in mice of lines 46 and 49, where they corresponded to 1–2% of the level of endogenous β -globin mRNA (or ~700 molecules of hybrid β -globin mRNA per reticulocyte). Because these mice contained only one gene copy per diploid genome, the level of expression per gene copy was 2–4% of the normal level. Erythroid tissues from mice of lines 75A and 77 contained considerably lower levels of hybrid β -globin mRNA (Table 1).

The transcripts synthesized from the hybrid β -globin gene in erythroid cells of the transgenic mice were found, by primer extension analysis, to be correctly initiated and spliced. A short, ^{32}P -labelled primer from the third exon of the human β -globin gene was annealed to blood RNA from mice in lines 46 and 77, as well as to human β -globin mRNA. When the hybridized primer was extended with reverse transcriptase and dNTPs, the major primer extension products obtained with blood samples from transgenic mice and with human cord blood were indistinguishable in size (Fig. 3).

Gene transfer studies with murine erythroleukaemia (MEL) cells have led to the conclusion that DNA sequences closely

linked to, and perhaps within, the β -globin gene are sufficient for the activation of a transfected gene during the terminal events of erythroid cell differentiation^{4,11–13}. Our results allow this statement to be extended to include any additional *cis*-acting regulatory sequences which may be needed to activate the gene specifically in the erythroid cells of a developing transgenic mouse. This conclusion must, however, be qualified in several ways. First, maximal transcription of the β -globin gene may require additional regulatory sequences not included on the DNA fragment used in our experiments. The steady-state concentration of the hybrid mRNA in transgenic mouse erythroid cells was no more than a few per cent of the level of endogenous β -globin mRNA, suggesting that the foreign gene was transcribed at a relatively low rate (although an alternative interpretation—a relatively low stability of the hybrid message—has not been eliminated). The highest mRNA levels observed were similar to the mRNA levels (per gene copy) observed when cloned β -globin genes were introduced into MEL cells^{4,11–13}.

Second, although it seems that the β -globin gene need not be in its normal chromosomal locus to be expressed and regulated, its expression may depend on integration at a specific subset of the available chromosomal positions. Several of the transgenic lines analysed do not express the hybrid β -globin genes in either erythroid or non-erythroid tissues, although they contain at least one intact copy of the gene (Table 1; data not shown). The β -globin gene is not unique in this regard, as several other genes, including metallothionein fusion genes^{7,14} and a mouse α -fetoprotein gene¹⁵, are expressed in only a subset of transgenic mouse lines. Occasional cases of low-level expression in inappropriate tissues (for example, mouse lines 15, 49 and 75A; refs 3, 14, 16, 17) may be another consequence of the integration of microinjected genes at abnormal chromosomal positions.

Given the present results, it is surprising that many other cloned α - and β -globin genes are not expressed in erythroid tissues when introduced into the mouse germ line (refs 3, 18, 19; T. Townes *et al.*, personal communication; S. Rusconi,

Table 1 Transgenic mouse lines

Mouse line	DNA injected	Gene copy no.*	Tissue	Hybrid β -globin mRNA in erythroid tissues		
				(No. of mice analysed)	RNA fraction†	% Of total β -globin mRNA§
46	5-kb <i>Cla</i> I– <i>Bgl</i> II fragment	1	Blood	(1)	8×10^{-5}	1.6
			Bone marrow	(3)	5×10^{-6}	0.6
			Spleen†	(1)	5×10^{-6}	0.6
49	5-kb <i>Cla</i> I– <i>Bgl</i> II fragment	1	Blood	(1)	10^{-4}	2.0
			Bone marrow	(1)	10^{-5}	1.2
			Spleen†	(1)	1.5×10^{-7}	0.015
75A	5-kb <i>Cla</i> I– <i>Bgl</i> II fragment	6	Blood	(2)	10^{-6}	0.02
			Bone marrow	(4)	2×10^{-7}	0.02
			Spleen†	(1)	1.5×10^{-7}	0.015
77	5-kb <i>Cla</i> I– <i>Bgl</i> II fragment	3	Blood	(2)	4×10^{-6}	0.08
			Bone marrow	(4)	8×10^{-7}	0.08
			Spleen†	(2)	10^{-6}	0.1
43	5-kb <i>Cla</i> I– <i>Bgl</i> II fragment	1	No expression detected			
47	5-kb <i>Cla</i> I– <i>Bgl</i> II fragment	1				
53	5-kb <i>Cla</i> I– <i>Bgl</i> II fragment	1				
11	Plasmid pMH β	9				
16	Plasmid pMH β	180	Very low expression ($< 10^{-7}$) in all tissues			
15	Plasmid pMH β	3				

Approximately 400–800 circular dimers of the entire plasmid, or 10–30 linear molecules of the *Cla*I–*Bgl*II fragment, were microinjected into the pronuclei of (C57BL/6J $\times$ CBA/J) F₂ mouse zygotes. All animals examined were transgenic progeny hemizygous for the hybrid β -globin gene.

* Number of copies of the 3.4-kb *Pst*I fragment per diploid genome in transgenic progeny, determined by densitometry of an autoradiogram of a Southern blot (not shown).

† Animals were treated with phenylhydrazine to induce splenic erythropoiesis, as described previously^{3,10}.

‡ Fraction of total RNA consisting of hybrid β -globin mRNA, determined by 3' S₁ nuclease analysis of mouse tissue RNAs, using human cord blood RNA containing a known concentration of human β -globin mRNA as a standard (see, for example, Fig. 2).

§ RNA fraction as a percentage of endogenous mouse β -globin RNA fraction in the same tissue. Values used for mouse β -globin RNA fractions were: blood, 5×10^{-3} ; bone marrow, and spleen of phenylhydrazine-treated mice, 9×10^{-4} . These values were determined by RNA titration analysis using a single-stranded probe of known specific activity²⁵.

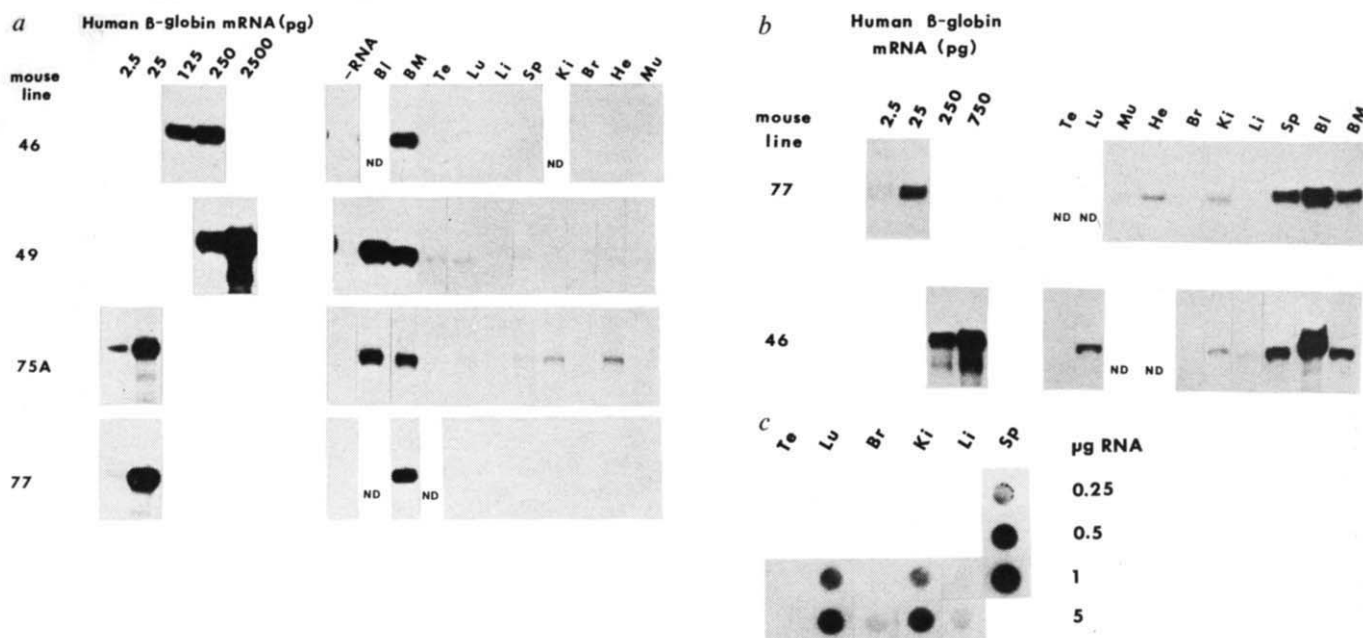


Fig. 2 Specific expression of the hybrid mouse/human β -globin gene in erythroid tissues. **a**, S_1 nuclease analysis of hybrid β -globin mRNA in mouse lines 46, 49, 75A and 77. Total RNA samples from the indicated tissues of a second- or third-generation mouse in each line were analysed by hybridization with a 3'-end-labelled probe derived from the 3' end of the human β -globin gene, as described elsewhere⁴. For comparison, hybridizations were also performed with human cord blood RNA containing a known amount of human β -globin mRNA. The hybrids were digested with S_1 nuclease and the products fractionated on a 6% polyacrylamide gel containing 7 M urea. Only those regions of the autoradiograms showing the 212-nucleotide probe fragment protected by hybrid mouse/human β -globin mRNA or human β -globin mRNA are shown. The intensities of hybridization signals from the four mice cannot be compared directly because the autoradiograms were exposed for different lengths of time. -RNA, hybridization with only yeast RNA carrier (25 μ g); BL, blood; BM, bone marrow; Te, testis; Lu, lung; Li, liver; Sp, spleen; Ki, kidney; Br, brain; He, heart; Mu, skeletal muscle. 10 μ g of RNA from blood and 25 μ g of RNA from all other tissues were used for each hybridization reaction. ND, not determined for the animal whose analysis is shown here. The human 3' probe does not cross-hybridize with endogenous mouse β -globin mRNA in the conditions used (data not shown). **b**, S_1 nuclease analysis of hybrid β -globin mRNA in tissues of mice treated with phenylhydrazine. A mouse from line 46 and one from line 77 were treated with phenylhydrazine to induce anaemia. Total RNA (25 μ g) from each of the indicated tissues was used for hybridization and S_1 nuclease analysis. The specific hybridization signal (212-nucleotide protected fragment) obtained with RNA from each tissue is shown in **a**. Note the greatly increased level of hybrid mouse/human β -globin mRNA in the spleen RNAs, compared with spleen RNAs from mice in lines 46 and 47 which were not treated with phenylhydrazine. **c**, Dot-blot analysis of endogenous β -globin mRNA contamination in non-erythroid tissues. To estimate the relative degree of blood contamination in non-erythroid tissues, endogenous β -globin mRNA was measured in RNA samples from a phenylhydrazine-treated mouse in line 46 (the same mouse as analysed in **b**). RNAs from the indicated tissues were denatured with formaldehyde, spotted onto a nitrocellulose filter²⁶, and the filter was hybridized with a 32 P-labelled mouse β -major cDNA clone²⁷. The two non-erythroid tissues with the highest level of blood contamination (lung and kidney) are the same two tissues that contain the most hybrid β -globin mRNA (**b**).

Methods: Mice were killed by cervical dislocation, organs were dissected and total RNA was isolated as described previously^{3,28}. Blood was obtained by heart puncture, and cells were washed once in cold phosphate-buffered saline containing 10 U ml⁻¹ heparin before isolation of total RNA. The 0.75-kb *EcoRI*-*PstI* fragment of the human β -globin gene was 3'-end-labelled with 32 P, and strand-separated on a polyacrylamide gel²⁹ as described^{3,4}. The specific activity of the probe was $2-4 \times 10^7$ c.p.m. μ g⁻¹, and 4 ng of probe were used for each hybridization reaction. In these conditions of probe excess, the hybridization signal observed (212-nucleotide protected fragment) is proportional to the β -globin mRNA concentration in each reaction. Hybridization was performed at 45 °C for 12-16 h, in 80% formamide, 0.4 M NaCl, 0.04 M PIPES, pH 6.8, 1 mM EDTA. Hybrids were mixed with denatured salmon sperm DNA (50 μ g ml⁻¹) and digested with 400 U ml⁻¹ S_1 nuclease (Sigma) at 15 °C for 1 h. The concentration of β -globin mRNA in the human cord blood RNA was determined (by R_0t analysis; data not shown) to be 1%, in agreement with published estimates of the globin mRNA content of reticulocytes³⁰.

personal communication). There are several factors that may be responsible for the successful expression of the hybrid β -globin gene in our experiments. First, it is possible that the use of mouse DNA sequences in the hybrid gene construct provided an essential regulatory element that is absent or divergent in the mammalian globin genes used previously. Second, the two transgenic lines which express the hybrid β -globin gene at the highest levels contain a single copy of the gene, rather than the multiple, tandem copies usually observed. The arrangement of exogenous globin genes in tandem arrays may provide an inferior substrate for transcriptional regulation. A different explanation, which we favour, is that plasmid or bacteriophage λ sequences may have an inhibitory effect on β -globin gene expression in transgenic mice; this interpretation is suggested by the fact that all four mouse lines which showed tissue-specific expression contained the hybrid β -globin gene with most of the plasmid sequences removed (Table 1). Furthermore, most of the cloned globin genes that were not expressed in previous experiments were

introduced together with prokaryotic vector sequences. This hypothesis remains to be tested definitively.

The results presented here, as well as recent studies demonstrating tissue-specific expression of other microinjected genes^{15,16,20-23}, indicate that the transgenic mouse system will be useful for elucidating the mechanisms by which genes are activated in specific cell types during development. One important aspect of globin gene regulation which this technique should allow us to study experimentally is the switch from embryonic to adult haemoglobin synthesis during mouse embryogenesis²⁴. An analysis of the timing of expression of the mouse/human hybrid β -globin gene has shown that the gene is turned on at the appropriate stage of development for an adult β -globin gene in the mouse (J.M., K.C. and F.C., in preparation). Experiments with additional cloned globin genes, such as embryonic genes or embryonic/adult fusion genes, may allow us to identify *cis*-acting DNA sequences involved in the control of haemoglobin switching.

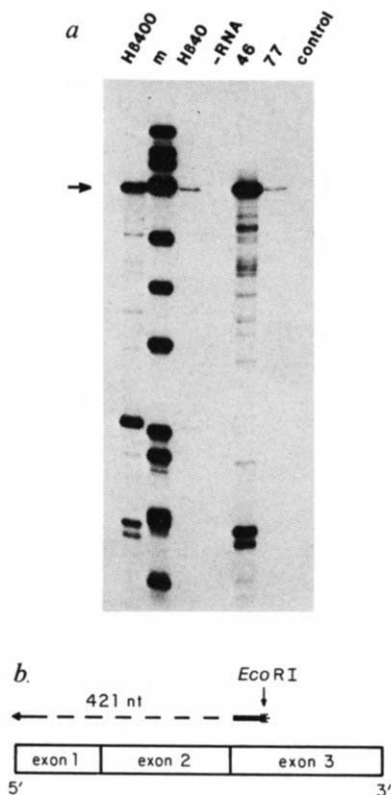


Fig. 3 Analysis of the 5' terminus of mouse/human hybrid β -globin mRNA by primer extension. A 32 P-labelled single-stranded primer, consisting of a 48-nucleotide *Bst*NI-*Eco*RI fragment from the third exon of the human β -globin gene, was hybridized with human cord blood RNA or blood RNA from transgenic or control mice. The primer was extended with reverse transcriptase and the products denatured and analysed by electrophoresis on a 6% polyacrylamide gel containing 7 M urea. **a**, Autoradiogram showing analysis of the following RNA samples: HB400 and HB40, human cord blood RNA containing 400 pg and 40 pg, respectively, of β -globin mRNA; m, molecular weight markers (ϕ X174 DNA digested with *Hinf*I); -RNA, yeast RNA carrier alone (25 μ g); 46, total blood RNA (25 μ g) from a mouse in line 46; 77, total blood RNA (25 μ g) from a mouse in line 77; control, total blood RNA (25 μ g) from a mouse not carrying the hybrid mouse/human β -globin gene. The position of the major reverse transcription products expected (418 nucleotides for human β -globin mRNA and 421 nucleotides for the hybrid β -globin mRNA) is indicated by the arrow. No signal was observed when the same reaction was performed with normal mouse blood RNA, showing that the primer is detecting hybrid mouse/human β -globin mRNA rather than endogenous β -globin mRNA in transgenic mouse blood. **b**, Diagram of the primer extension assay. The heavy line represents the labelled primer and the dotted line the product of reverse transcription after hybridization with human or hybrid mouse/human β -globin mRNA. Preparation of the primer, hybridization and reverse transcription were performed as described in refs 4 and 31, except that 0.4 ng of primer was used for each reaction.

We thank Tom Maniatis and Richard Axel for helpful comments on the manuscript. K.C. was supported by a fellowship from NATO. F.C. is a recipient of an Irma T. Hirsch Career Scientist Award. This work was supported by grants to F.C. from the NIH and the National Foundation—March of Dimes.

Received 20 September; accepted 18 December 1984.

- Collins, F. S. & Weissman, S. M. *Prog. Nucleic Acids Res.* **31**, 315-462 (1984).
- Costantini, F. & Lacy, E. *Nature* **294**, 92-94 (1981).
- Lacy, E., Roberts, S., Evans, E. P., Burtenshaw, M. D. & Costantini, F. D. *Cell* **34**, 343-358 (1983).
- Chao, M. V., Mellon, P., Charnay, P., Maniatis, T. & Axel, R. *Cell* **32**, 483-493 (1983).
- Gordon, J., Scangos, G. A., Plotkin, D. J., Barbosa, J. A. & Ruddle, F. H. *Proc. natn. Acad. Sci. U.S.A.* **77**, 7380-7384 (1980).

- Wagner, E. F., Stewart, T. A. & Mintz, B. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5016-5020 (1981).
- Brinster, R. L. *et al. Cell* **27**, 223-231 (1981).
- Berk, A. J. & Sharp, P. A. *Cell* **12**, 721-732 (1977).
- Weaver, R. & Weissman, C. *Nucleic Acids Res.* **7**, 1175-1193 (1979).
- Conkie, D., Kleiman, L., Harrison, P. R. & Paul, J. *Expl. Cell Res.* **93**, 315-324 (1975).
- Wright, S., deBoer, E., Grosfeld, F. G. & Flavell, R. A. *Nature* **305**, 333-336 (1983).
- Charnay, P. *et al. Cell* **38**, 251-263 (1984).
- Wright, S., Rosenthal, A., Flavell, R. & Grosfeld, F. *Cell* **38**, 265-273 (1984).
- Palmiter, R. D., Norstedt, G., Gelinas, R. E., Hammer, R. E. & Brinster, R. L. *Science* **222**, 809-814 (1983).
- Krumlauf, R., Hammer, R., Tilghman, S. & Brinster, R. *Molec. cell. Biol.*, (submitted).
- McKnight, G. S., Kuenzel, E. A., Hammer, R. E. & Brinster, R. L. *Cell* **34**, 335-341 (1983).
- Jaenisch, R. *et al. Cell* **24**, 519-529 (1981).
- Stewart, T. A., Wagner, E. F. & Mintz, B. *Science* **217**, 1046-1048 (1982).
- Humphries, R. K. *et al. in Eucaryotic Gene Expression* (eds Kumar, A., Goldstein, A. L. & Bahouney, G. V.) (Plenum, New York, 1983).
- Brinster, R. L. *et al. Nature* **306**, 332-336 (1983).
- Storb, U., O'Brien, R. L., McMullen, M. D., Gollahon, K. A. & Brinster, R. L. *Nature* **310**, 238-240 (1984).
- Grosschedl, R., Weaver, D., Baltimore, D. & Costantini, F. *Cell* **38**, 647-658 (1984).
- Swift, G. H., Hammer, R. E., MacDonald, R. J. & Brinster, R. L. *Cell* **38**, 639-646 (1984).
- Russell, E. S. *Adv. Genet.* **20**, 357-459 (1979).
- Scheller, R. H., Costantini, F. D., Kozlowski, M. R., Britten, R. J. & Davidson, E. H. *Cell* **15**, 189-203 (1978).
- Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201-5205 (1980).
- Rougeon, F. & Mach, B. *Gene* **1**, 229-239 (1977).
- Chirgwin, J., Przybyla, A., MacDonald, R. & Rutter, W. J. *Biochemistry* **18**, 5294-5299 (1979).
- Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65**, 499-560 (1980).
- Humphries, S., Windass, J. & Williamson, R. *Cell* **7**, 267-277 (1976).
- Treisman, R., Proudfoot, N. J., Shander, M. & Maniatis, T. *Cell* **29**, 903-911 (1982).

Partial deficiency of erythrocyte spectrin in hereditary spherocytosis

Peter Agre*†, James F. Casella‡, William H. Zinkham‡, Campbell McMillan§ & Vann Bennett†

Departments of *Medicine, †Cell Biology/Anatomy and ‡Pediatrics, Johns Hopkins School of Medicine, Baltimore, Maryland 21205, USA

§ Department of Pediatrics, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514, USA

Hereditary spherocytosis (HS) is a common, clinically heterogeneous haemolytic anaemia^{1,2} in which the primary erythrocyte defect is believed to be some abnormality in the spectrin-actin membrane skeleton^{3,4}, leading to loss of surface membrane⁵. Recessively inherited spectrin deficiency with extreme erythrocyte fragility and spherocytosis has been identified in certain mutant mice^{6,7} and two severely anaemic humans⁸. Although suspected, deficiency of spectrin has not been demonstrated in less severe forms of human HS⁹. We now report the quantitation of erythrocyte spectrin by radioimmunoassay. We found that normal erythrocytes contained 240,000 copies of spectrin heterodimer, whereas erythrocytes from 14 patients with a variety of types of HS were all partially deficient in spectrin (range 74,000-200,000 copies), the magnitude of the deficiency correlating with the severity of the disease. Spectrin deficiency of varying degrees is common in HS and probably represents the principal structural defect leading to loss of surface membrane.

Analysis of Coomassie blue-stained SDS-polyacrylamide electrophoresis (PAGE) gels has revealed deficiencies of specific erythrocyte membrane proteins in certain inherited haemolytic anaemias¹⁰. We have subsequently found that erythrocytes from humans with mild HS have very subtle reductions in spectrin relative to band 3. Figure 1 shows a Coomassie blue-stained SDS-PAGE slab with erythrocyte membranes prepared from a normal individual and four patients with HS. Determination of relative protein concentrations by the pyridine dye elution method¹¹ showed the amount of spectrin relative to band 3 to be reduced in the HS samples (Fig. 1 legend). Similar membrane analyses were conducted on erythrocytes from more than 100 individuals, including HS patients from 28 different families. We found that erythrocyte membranes from 39 of 43 HS patients showed small but consistent reductions in spectrin relative to band 3, whereas unaffected relatives, patients with other types of anaemia (including warm-reacting autoimmune haemolytic

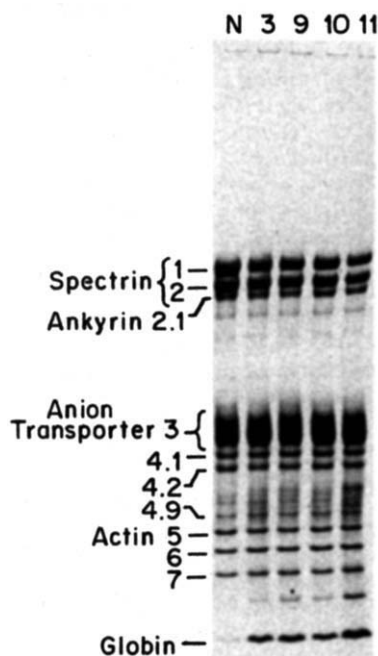


Fig. 1 Coomassie blue-stained SDS-PAGE slab of membranes from a normal individual and four HS patients. Freshly drawn venous blood was washed three times in phosphate-buffered saline (PBS) with removal of the buffy coat. Erythrocytes were lysed and washed repetitively at 0°C with 7.5 mM sodium phosphate, 1 mM Na-EDTA, 0.2 mM phenylmethylsulphonyl fluoride as described previously²⁷. Approximately 25 µg of membrane protein from samples obtained from each of several patients and a control was applied to the top of a 3.5–17% exponential gradient polyacrylamide slab gel and electrophoresed for 3 h at 240 V (ref. 28). Membranes in lane N are from a normal individual and membranes in the other lanes are from patients listed in Table 1. Relative protein concentrations in the spectrin regions of each lane were compared with that in the band 3 region by the pyridine dye elution method¹¹ and were analysed as the ratios of absorbance at 605 nm of spectrin and band 3: normal = 1.12; patients: 3 = 0.74; 9 = 0.84; 10 = 0.84; 11 = 0.65.

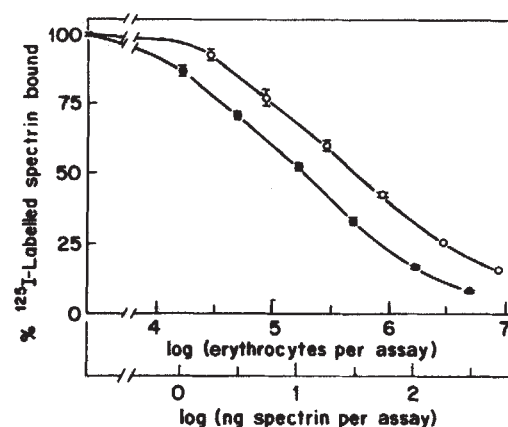


Fig. 2 Radioimmunoassay using ¹²⁵I-labelled spectrin which is displaced from anti-spectrin antibodies adsorbed onto protein A-bearing staphylococci by spectrin purified from normal erythrocytes (○) or by detergent-solubilized erythrocytes (●). **Methods.** Spectrin was purified from normal erythrocytes²⁷, and the precise protein content was determined from the ultraviolet absorbance spectrum and the extinction coefficient (ref. 29), $E_{1\text{cm}}^{1\%}$ 10.7. Pure spectrin was radiolabelled with Na¹²⁵I to a specific activity of 2×10^7 c.p.m. per µg as described by Hunter and Greenwood³⁰ and stored at 0°C at a concentration of $13 \mu\text{g ml}^{-1}$ in radioimmunoassay buffer (150 mM NaCl, 10 mM sodium phosphate, 1 mM EDTA, 1 mM NaN₃, 1% (v/v) Triton X-100, pH 7.5, also containing 1 mg ml⁻¹ bovine serum albumin and 5 µg ml⁻¹ pancreatic trypsin inhibitor). The immunoabsorbent was prepared from washed protein A-bearing staphylococci and serum from rabbits immunized with human spectrin as described elsewhere³¹. Erythrocytes were washed three times in PBS with removal of the buffy coat and suspended to a haematocrit of 10%. Aliquots of this suspension were removed and counted with a Coulter model B instrument. Aliquots of the erythrocyte suspensions, pure spectrin and ¹²⁵I-labelled spectrin were separately dissolved and incubated at 60°C for 10 min in PBS containing 1% (w/v) SDS and then diluted at least 40-fold into radioimmunoassay buffer. The assay was performed in 12 × 75-mm polystyrene tubes containing 0.2 ml of radioimmunoassay buffer with ~100,000 c.p.m. of ¹²⁵I-spectrin, varying dilutions of erythrocyte lysate or pure spectrin and a 0.5% (v/v) suspension of anti-spectrin-staphylococci (diluted ~1:9 with washed staphylococci to reduce the capacity of the immunoabsorbent to precipitate only 40% of the total ¹²⁵I-spectrin). Samples were shaken gently overnight at 4°C, then washed with 4 ml of 2 M urea, 1% (v/v) Triton X-100, 0.1 M glycine and the pellets were collected by centrifugation (20 min at 3,500g) and analysed for ¹²⁵I. The final SDS concentration was never more than 0.025% (w/v) and, in the presence of 1% (v/v) Triton X-100, did not interfere with ¹²⁵I-spectrin binding. Binding of ¹²⁵I-spectrin to control immunoabsorbents, prepared from preimmune rabbit serum and staphylococci, was negligible and therefore not subtracted. Points represent means ± s.e.m. for triplicates. The content of spectrin in solubilized erythrocytes was determined by calculating the number of cells and the quantity of pure spectrin producing 50% inhibition of ¹²⁵I-spectrin binding by linear regression of data points in the linear portions of the profiles. A radioimmunoassay using ¹²⁵I-labelled 43,000- M_r cytoplasmic fragment of band 3, prepared as described by Bennett²⁷, and immunoabsorbent, prepared from affinity-purified anti-43,000- M_r fragment IgG and protein A-bearing staphylococci, was performed similarly.

anaemia) and other controls showed no such reduction. Although these analyses indicate that a relative spectrin deficiency commonly exists in HS, they are only semi-quantitative, as this method does not allow determination of the actual number of copies of spectrin per erythrocyte.

We therefore developed a radioimmunoassay to quantify the number of copies of spectrin per erythrocyte. This assay is performed on washed erythrocytes using purified control spectrin as a standard and ¹²⁵I-labelled pure spectrin as a probe (Fig. 2). Incubation of a known number of washed erythrocytes in 1% SDS at 60°C results in complete unfolding of membrane proteins, allowing accurate measurement of the actual number of copies per cell by radioimmunoassay¹². The SDS-lysed erythrocytes were diluted into buffer containing Triton X-100 and albumin to quench the denaturing effect of SDS. Spectrin in these lysates and purified spectrin each competed with ¹²⁵I-labelled spectrin for binding to immobilized anti-spectrin antibodies, and the profiles of displacement by erythrocyte lysates and by pure spectrin were virtually parallel (Fig. 2). The concentration of purified spectrin and the number of erythrocytes producing 50% displacement of ¹²⁵I-labelled spectrin were determined by measurements on erythrocytes from each of three normal adults on multiple occasions, and an average of 240,000 copies of spectrin heterodimer per normal erythrocyte was calculated (range 225,000–258,000), reasonably close to the 216,000 copies estimated from Coomassie blue-stained SDS-PAGE gels¹³.

The radioimmunoassay can also be modified to measure band 3 content. However, purified band 3 is extremely hydrophobic and unstable, and therefore is neither a good protein to label with ¹²⁵I nor a stable source of protein standard. Therefore, it was necessary to use an ¹²⁵I-labelled cytoplasmic fragment of band 3 (relative molecular mass (M_r) 43,000) as a stable, water-soluble probe¹⁴ and normal control erythrocytes as a standard for comparison with HS erythrocytes.

Spectrin and band 3 radioimmunoassays were performed in parallel on erythrocytes from multiple controls as well as HS

patients. The band 3 radioimmunoassay was used, as band 3 is an integral membrane protein, 60–80% of which is not linked directly to the membrane skeleton via ankyrin¹⁵, and it was expected that the unlinked fraction would be relatively preserved even in membranes deficient in membrane skeleton proteins. Table 1 shows the results of these analyses. It is clear that normal individuals of different ages, individuals who had undergone splenectomy for reasons other than anaemia, and patients without HS who had microcytosis and reticulocytosis all had a similar number of copies of spectrin per erythrocyte. Of 14 HS patients studied who had dominant or non-dominant patterns of inheritance and clinical severities ranging from mild to nearly lethal, all had significant reductions in the number of copies of spectrin per erythrocyte. The reductions varied between patients but correlated with the clinical estimation of disease severity, ranging from 200,000 copies per erythrocyte for the least affected to only 74,000 for the most seriously affected. The reduction in the number of copies of spectrin was highly reproducible when measured on multiple occasions on erythrocytes from the same HS patients (interassay variability $\leq \pm 4\%$). Erythrocyte spectrin content was also found to be similar within families when members with anaemias of comparable clinical severity were compared (members of families B, D and H). Note that the splenectomized member of family D and the relative who had a spleen had erythrocytes with similar spectrin contents. Members within families A and E who had anaemias of different clinical severities were found to have correspondingly different spectrin contents which reflected clinical severity.

Increased sensitivity to hypotonic lysis indicates a relative decrease in surface area to volume¹⁶ and is helpful in establishing diagnosis in HS¹. Blood samples from the patients listed in Table 1 were quantitatively analysed for shifts in hypotonic lysis profile, and this reflected both spectrin content and disease severity (Fig. 3). Although band 3 content was usually slightly reduced, it was generally less reduced than the spectrin content

and was much more variable. It is probable that band 3 content is somewhat more reduced before splenectomy because of the selective removal of certain regions of the membrane by the spleen, possibly related to the process referred to as splenic 'conditioning'. This idea is supported in family D, where the patient without a spleen had significantly more band 3, and higher band 3 contents were also noted in splenectomized non-anaemic individuals. Because of reductions in band 3, the apparent reduction in spectrin relative to band 3 determined from Coomassie blue-stained SDS-PAGE gels always underestimated the true corpuscular spectrin deficiency as measured by radioimmunoassay. The ratio of spectrin to band 3 determined from SDS-PAGE gels (see Fig. 1 legend) is similar to the quotient of the spectrin and band 3 radioimmunoassays (Table 1).

Erythrocytes from patients with all types of HS were found to be deficient in spectrin to varying degrees, whereas erythrocytes from several controls and patients with other types of anaemias were not. A common underlying defect such as partial spectrin deficiency provides a logical explanation for a clinically heterogeneous anaemia in which a reduction in membrane surface area is a recognized common feature. Several observations indicate that the deficiency of spectrin is a primary factor in the pathogenesis of spherocytes. To date, no case of spectrin deficiency has been reported without spherocytes being found in the peripheral blood. Furthermore, when spectrin and actin are stripped from normal erythrocyte membranes, the structure spontaneously disintegrates into tiny vesicles. A partial deficiency in spectrin may result in an underlying membrane skeleton which may not support adequately all regions of the lipid bilayer, leading to loss of small areas of unsupported lipid and untethered integral membrane proteins. If the fragility of HS membranes results primarily from deficiency of spectrin, it might be corrected by addition of spectrin; incorporation of spectrin into erythrocytes of the spectrin-deficient *sph/sph* mutant mice by exchange haemolysis led to partial correction

Table 1 Radioimmunoassay of spectrin and band 3 in erythrocytes

					Radioimmunoassay		
					Spectrin	Band 3	
					Copies per cell	% Of control	% Of control
Control individuals							
Normal adults, 3 persons					240,000	100	100
Normal child					235,000	98	102
Splenectomy without anaemia, 2 persons					241,000	100	119
Hypoplastic anaemia with ovalocytes					250,000	104	
Microcytic anaemia (iron deficiency)					238,000	99	
Microcytosis with reticulocytosis (Hb SC)					255,000	106	108
Patients with hereditary spherocytosis							
Family	Inheritance pattern*	Patient	Clinical severity†	Splenectomy			
A	Dominant	1	Mild		200,000	84	108
		2	Moderate	+	140,000	58	88
		3	Severe	+	122,000	51	86
B	Dominant	4	Mild		192,000	80	69
		5	Mild		187,000	78	69
C	Dominant	6	Mild	+	182,000	76	
D	Dominant	7	Moderate		166,000	69	87
		8	Moderate	+	168,000	70	104
E	Recessive	9	Severe	+	128,000	53	94
		10	Nearly lethal	+	108,000	45	79
F	Non-dominant	11	Nearly lethal	+	80,200	33	68
G	Non-dominant	12	Nearly lethal	+	75,800	32	66
H	Recessive	13‡	Nearly lethal	+	81,600	34	62
		14‡	Nearly lethal	+	74,400	31	69

* Dominant: one affected parent. Non-dominant: both parents clinically normal with no apparent consanguinity. Recessive: both parents clinically normal with known consanguinity.

† Mild: subclinically affected, no anaemia, reticulocytosis $\leq 2\%$, splenectomy considered optional. Moderate: anaemia compensated by reticulocytosis before splenectomy which was performed before age 10 yr, no anaemia or reticulocytosis post-splenectomy. Severe: incompletely compensated haemolytic anaemia requiring occasional transfusions before splenectomy performed before age 3 yr, reticulocytosis $> 2\%$ post-splenectomy. Nearly lethal: dependent on regular transfusions to maintain haematocrit $> 14\%$ before splenectomy performed in infancy, post-splenectomy reticulocytes 4–10% with haematocrit $\sim 30\%$ in patients 11–14.

‡ Previous evaluations of these patients have been reported.

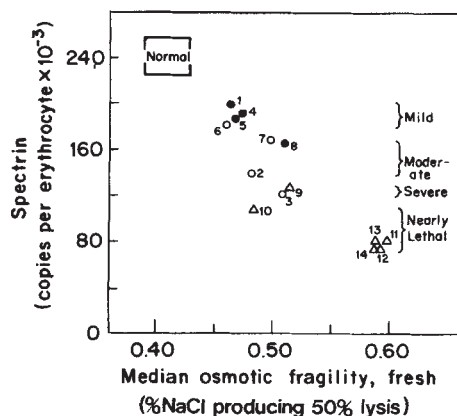


Fig. 3 Correlation of erythrocyte spectrin content and sensitivity to hypotonic lysis. Erythrocyte spectrin content was estimated by radioimmunoassay for each patient and is listed in Table 1: $\circ$, $\bullet$, dominant inheritance; Δ , non-dominant or recessive inheritance; $\circ$, Δ , previous splenectomy. The sensitivity to hypotonic lysis (osmotic fragility) was conducted on fresh blood samples mixed with PBS of concentrations ranging from 0.9% NaCl to distilled water¹. Median lysis was measured by interpolating between points to estimate the concentration of NaCl at which 50% of cells had lysed. The box at the upper left indicates the range of spectrin determinations on erythrocytes from multiple individuals (Table 1) and the range of median lysis as determined on erythrocytes from multiple normal adults and children.

of the mechanical fragility¹⁷. Reductions in other proteins that bind to spectrin would be expected secondary to deficiency in spectrin, and small but variable reductions in band 3 content were noted by radioimmunoassay as well as small reductions in ankyrin, band 4.1 and actin by analysis of SDS-PAGE gels⁸ (for example, Fig. 1). Band 3 deficiency is unlikely to be the primary defect in HS, as this correlated neither with clinical severity nor with osmotic fragility. Small reductions in ankyrin probably result from early degradation of uncomplexed ankyrin, although primary ankyrin defects or deficiencies may exist in certain HS families. A small reduction in band 4.1 is also unlikely to be a primary structural defect in HS, as a 50% deficiency in band 4.1 results, not in HS but in a subclinical form of ovalocytosis^{18,19}.

The spectrin deficiency in human HS probably results from many genetic defects, with each kindred carrying a different mutation. Probable abnormalities are likely to fall into two groups, a reduced spectrin synthesis due to abnormal gene regulation or unstable mRNA, and inherent protein defects in α - or β -spectrin subunits or ankyrin, leading to defective protein-protein associations, intracellular precipitation or increased susceptibility to proteolysis. Bodine and colleagues found spectrin-deficient spherocytic mouse mutants lacking ankyrin (*nb/nb*), with reduced spectrin α -subunit synthesis (*sph/sph*), synthesis of apparently unstable α -subunits (*sph^{na}/sph^{na}*) and reduced spectrin β -subunit synthesis (*ja/ja*)²⁰. Erythrocytes from three human families with dominantly inherited spherocytosis have been shown to possess two populations of spectrin, a normal population and a variant population binding defectively to band 4.1 (refs 21, 22). Members of the latter family have been found recently to have a reduction in spectrin relative to band 3 on SDS-PAGE gels (P. Becker and S. Lux, unpublished). The defect in the spectrin-4.1 interaction may result either in reduced spectrin incorporation into the membrane or increased degradation of membrane spectrin, and as expected, it was the variant population that was quantitatively reduced^{21,22}. Hereditary pyropoikilocytosis is a rare anaemia²³⁻²⁵ in which an inherent spectrin defect probably contributes directly to membrane instability¹⁰ but may also lead to secondary spectrin deficiency, while reduced binding of spectrin to ankyrin is

another mechanism which could lead to reductions in membrane spectrin (for example, ref. 26). Thus, it is likely that a large number of human genetic defects, although variable in degree, will have the common result of an overall deficiency in spectrin. The studies reported here indicate that spectrin deficiency, from whatever cause, will result in a haemolytic, spherocytic anaemia if the erythrocytes contain less than 200,000 copies of spectrin.

We thank the patients and their families for their generous and enthusiastic participation in this study. The work was supported by grants from the NIH: 1-R01-AM29808-04 to V.B., Clinical Investigator Awards to P.A. and J.F.C. and Research Career Development Award to V.B.

Received 21 September; accepted 12 December 1984.

1. Dacie, J. V. in *The Haemolytic Anemias: Congenital and Acquired* 2nd edn, 82-150 (Grune & Stratton, New York, 1960).
2. Young, L. E. *Trans. Ass. Am. Physns* **68**, 141-148 (1955).
3. Bennett, V. J. *cell. Biochem.* **18**, 49-65 (1982).
4. Cohen, C. M. *Semin. Hemat.* **20**, 141-168 (1983).
5. Cooper, R. A. & Jandl, J. H. *J. clin. Invest.* **48**, 736-744 (1969).
6. Greenquist, A. C., Shohet, S. B. & Bernstein, S. E. *Blood* **51**, 1149-1155 (1978).
7. Lux, S. E., Pease, B., Tomaselli, M. B., John, K. M. & Bernstein, S. E. in *Normal and Abnormal Red Cell Membranes* (eds Lux, S. E., Marchesi, V. T. & Fox, C. F.) 463-469 (Liss, New York, 1979).
8. Agre, P., Orringer, E. P. & Bennett, V. *New Engl. J. Med.* **306**, 1155-1151 (1982).
9. Knowles, W., Marchesi, S. & Marchesi, V. T. *Semin. Hemat.* **20**, 159-174 (1983).
10. Palek, J. & Lux, S. E. *Semin. Hemat.* **20**, 189-224 (1983).
11. Fenner, C., Traut, R. R., Mason, D. T. & Wikman-Coffelt, J. *Analyt. Biochem.* **63**, 595-602 (1975).
12. Bennett, V. *Nature* **281**, 597-599 (1979).
13. Steck, T. L. *J. Cell Biol.* **62**, 1-19 (1974).
14. Bennett, V. & Stenbuck, P. J. *biol. Chem.* **255**, 6424-6432 (1980).
15. Bennett, V. *Biochim. biophys. Acta* **689**, 475-484 (1982).
16. Castle, W. B. & Daland, G. A. *Am. J. Physiol.* **120**, 371-383 (1937).
17. Shohet, S. B. *J. clin. Invest.* **64**, 483-494 (1979).
18. Feo, C. J., Fischer, S., Piau, J. P., Grange, M. J. & Tchernia, G. *Nouv. Revue fr. Hémat.* **22**, 315-325 (1980).
19. Tchernia, G., Mohandas, N. & Shohet, S. B. *J. clin. Invest.* **68**, 454-460 (1981).
20. Bodine, D. M., Birkenmeier, C. S. & Barker, J. E. *Cell* **37**, 721-729 (1984).
21. Goodman, S. R., Shiffer, K. A., Casoria, L. A. & Eyster, M. E. *Blood* **60**, 779-784 (1982).
22. Wolfe, L. C., John, K. M., Falcone, J. C., Byrne, A. M. & Lux, S. E. *New Engl. J. Med.* **307**, 1367-1374 (1982).
23. Lawler, J., Liu, S.-C., Palek, J. & Prchal, J. J. *J. clin. Invest.* **70**, 1019-1030 (1982).
24. Knowles, W. J. *et al. J. clin. Invest.* **71**, 1867-1877 (1983).
25. Palek, J., Liu, S.-C., Liu, P. Y., Prchal, J. & Castleberry, R. P. *Blood* **57**, 130-139 (1981).
26. Zail, S. S. & Coetzer, T. L. *J. clin. Invest.* **74**, 753-762 (1984).
27. Bennett, V. *Meth. Enzym.* **96**, 313-324 (1983).
28. Fairbanks, G., Steck, T. L. & Wallach, D. F. H. *Biochemistry* **10**, 2606-2617 (1971).
29. Kam, Z., Josephs, R., Eisenberg, H. & Gratzner, W. B. *Biochemistry* **16**, 5568-5572 (1977).
30. Hunter, W. & Greenwood, F. *Nature* **194**, 495-496 (1962).
31. O'Keefe, E. & Bennett, V. *J. biol. Chem.* **255**, 561-568 (1980).

Corrigendum

High rates of *in vitro* synthesis of 1,4- β -D-glucan in cell-free preparations from *Phaseolus aureus*

T. Callaghan & M. Benizman
Nature **311**, 165-167 (1984)

This letter described conditions for high rates of *in vitro* synthesis of 1,4- β -glucan in membrane preparations derived from *Phaseolus aureus*. This was achieved with extracts prepared in polyethylene glycol 4000 and assayed in the presence of Mg^{2+} , Ca^{2+} and cellobiose. The 24% KOH-insoluble glucan product, formed from UDP ^{14}C -glucose, has been characterized as 1,4- β -D-glucan by several criteria, including digestion by purified exocellobiohydrolase and endo-1,4- β -glucanase, methylation analysis and periodate oxidation.

During further studies on the role of cellobiose in this *in vitro* system, more recent analyses of the product (in collaboration with Dr D. P. Delmer) have now revealed largely the formation of 1,3-glucan from UDP-glucose. This finding is puzzling and it is not yet clear why, in our early studies, 1,4-linked glucan was produced, whereas later, under the same conditions, the product was found to contain only 1,3 linkages.

Work is in progress to clarify exactly what conditions are required to obtain reproducible synthesis of cellulose *in vitro* with plant systems.

Pheromone-processing protease of the yeast *Saccharomyces cerevisiae*

A RECENT paper by Mizuno and Matsuo¹ reports the purification from the yeast *Saccharomyces cerevisiae* of a protease with specificity for cleaving at a pair of basic residues within several peptide substrates. The potential importance of such a finding stems from the fact that virtually all eukaryotic peptide hormones and neurosecretory peptides are released from their precursor polypeptides by scission at pairs of basic residues that flank the mature hormone sequences². Indeed, even in a simple eukaryotic microbe like yeast, secreted biologically active peptides, like the mating pheromone α -factor³⁻⁵ and killer toxin^{6,7}, are excised from their precursors by initial cleavages at pairs of basic residues, namely Lys-Arg.

The activity characterized by Mizuno and Matsuo¹ is soluble and produces Tyr-Gly-Gly-Phe-Leu-Arg (abbreviated leu-enkephalin-Arg) from a larger synthetic substrate (leu-enkephalin-Arg-Arg-Phe-Ala-NH₂). On the basis of this finding, but with no results relevant to the biology of yeast, Mizuno and Matsuo termed this enzyme 'pro-pheromone-convertase Y' and implied that it may be involved in proteolytic maturation of yeast pheromone precursors¹.

We find this reasoning and methodology to be flawed. Recently, we described a mutation (*kex2*) which blocks the proteolytic processing of both prepro- α -factor and prepro-killer toxin and have isolated the normal *KEX2* gene by recombinant DNA methodology⁸. We demonstrated that *kex2* mutants lack a membrane-associated protease with specificity for cleaving on the carboxyl side of a pair of basic residues (not between them). Furthermore, cells carrying the *KEX2* gene on a multi-copy plasmid greatly overproduce this protease. We have observed (ref. 8 and R. Fuller, unpublished results) that the *KEX2* protease has a strictly neutral pH optimum, is completely Ca²⁺-dependent, is inhibited by heavy metals (for example, Zn²⁺, Cu²⁺ and Cd²⁺), by thiol-modifying reagents and leupeptin, but is not sensitive to inactivation by serine protease inhibitors (for example, phenylmethylsulphonyl fluoride, PMSF). In contrast, the activity described by Mizuno and Matsuo has a broad pH optimum, is insensitive to chelators, resistant to sulphhydryl-directed reagents and unaffected by leupeptin, but is inhibited by several classical serine protease inhibitors (including PMSF)¹. Finally, the DNA sequence of the *KEX2* gene (A. Brake, unpublished results) predicts a polypeptide of relative molecular mass (*M_r*) 90,000 with a single, striking, membrane-spanning domain; the protein purified by

Mizuno and Matsuo¹ was reported to be of *M_r* ~43,000. Clearly, the *KEX2* protease and the protein described by Mizuno and Matsuo¹ are quite different enzymes.

The yield and specific activity of the preparation obtained by Mizuno and Matsuo were not given in their report. As best as can be calculated from the data provided, the final yield was only 2% or less of the initial activity. Given that one of the major neutral PMSF-sensitive vacuole-associated proteases of yeast, proteinase B, has been obtained as a soluble polypeptide of *M_r* ~41,000 when no precautions were taken to prevent proteolysis during purification⁹ (Mizuno and Matsuo added no protease inhibitors to their buffers for obvious reasons), and considering that purified proteinase B will cleave a synthetic substrate, Leu-Trp-Met-Arg-Phe-Ala (somewhat similar to that used by Mizuno and Matsuo), between the Arg and Phe¹⁰, it is possible that the activity described by Mizuno and Matsuo is a proteolytic fragment of proteinase B. This possibility is testable readily because there are available both antibodies directed against proteinase B¹¹ and mutations in its structural gene (*PRB1*)¹². Also, it should be noted that Mizuno and Matsuo generated cell homogenates by lysing whole cells with Zymolyase 60,000, a commercial preparation of secreted hydrolytic enzymes from the bacterium *Oerskovia xanthineolytica* (formerly classified as *Arthobacter luteus*). Hence, the activity described by Mizuno and Matsuo and attributed to yeast could actually result from contamination by the PMSF-sensitive bacterial protease known to be present in preparations of Zymolyase^{13,14}. Again, this possibility could have been eliminated if the Zymolyase treatment had been performed in osmotically stabilizing medium to generate spheroplasts and if the spheroplasts had been washed thoroughly before cell lysis.

J. THORNER

Department of Microbiology
and Immunology,
University of California,
Berkeley, California 94720, USA

- Mizuno, K. & Matsuo, H. *Nature* **309**, 558-560 (1984).
- Herbert, E. & Uhler, M. *Cell* **30**, 1-2 (1982).
- Kurjian, J. & Herskowitz, I. *Cell* **30**, 933-943 (1982).
- Julius, D., Blair, L., Brake, A., Sprague, G. & Thorner, J. *Cell* **32**, 839-852 (1983).
- Julius, D., Schekman, R. & Thorner, J. *Cell* **36**, 309-318 (1984).
- Bostian, K. *et al.* *Cell* **36**, 741-751 (1984).
- Skipper, N., Thomas, D. & Lau, P. *EMBO J.* **3**, 107-111 (1984).
- Julius, D., Brake, A., Blair, L., Kunisawa, R. & Thorner, J. *Cell* **37**, 1075-1089 (1984).
- Ulane, R. & Cabib, E. *J. biol. Chem.* **251**, 3367-3374 (1976).
- Kominami, E., Hoffschulte, H., Leuschel, L., Maier, K. & Holzer, H. *Biochim. biophys. Acta* **661**, 136-141 (1981).
- Mechler, B., Müller, M., Müller, H., Meussdoerfer, F. & Wolf, D. *J. biol. Chem.* **257**, 11203-11206 (1982).
- Zubenko, G., Mitchell, A. & Jones, E. W. *Genetics* **96**, 137-146 (1980).
- Scott, J. & Schekman, R. *J. Bact.* **142**, 414-423 (1980).
- Scott, J. thesis, Univ. California, Berkeley (1980).

MIZUNO AND MATSUO REPLY—As observed in three distinct precursors of enkephalin, pro-opiocrortin and pro-enkephalin A and B, the enkephalin units to be processed are flanked by paired basic residues such as Lys-Lys, Lys-Arg, Arg-Arg and Arg-Lys, which are thought to be a typical processing signal. This suggests strongly that proteases specific to paired basic residues may be involved in enkephalin processing. Our knowledge of the processing events from the precursor to the mature peptides, however, is limited and so far, candidate enzymes have not been identified^{1,2}. Hence, the suggestion that the proteolytic processing at the paired basic signals in the precursors occurs by sequential actions of trypsin-like and carboxypeptidase B-like proteases is only tentative.

S. cerevisiae α -cells synthesize and secrete α -mating factor, which, when processed from a larger precursor, involves cleavage at a paired basic signal, that is, Lys-Arg. Therefore, we have chosen these cells as a simple model system for the study of enkephalin processing and have identified in the cell lysates a novel protease which recognizes specifically and cleaves the peptide bond between paired basic residues³. The enzyme, of *M_r* ~43,000, cleaves various substrates related to pro-enkephalins between paired basic residues, but not at single basic residues. Its unique substrate specificity suggests that the enzyme is involved in pro-pheromone processing³. In order to clarify the processing mechanism, it is important that the candidate proteases be characterized in the various peptide-hormone-producing cells, even though it is unknown whether the enzyme really participates in the processing. Several investigators including us have looked for pro-hormone-converting enzymes in the secretory granules from anglerfish pancreatic islets⁴, rat neurointermediate lobe⁵ and bovine adrenomedullary gland^{6,7}. Protease activities converting pro-insulin, pro-glucagon, pro-somatostatin and pro-opiocrortin to the corresponding mature hormones were found. Our enzyme purified from yeast, however, is the only protease with specificity for a paired basic signal that has ever been isolated to homogeneity. We are convinced that our approach, which is distinct from Thorner's⁸, is necessary for elucidating the as yet unknown mechanism of pro-hormone processing.

We believe that our enzyme is neither a proteolytic fragment of proteinase B nor a contaminant from Zymolyase. Proteinase B is inhibited by 90% by thiol reagent, *p*-chloromercuribenzoic acid (pCMB), at 0.93 μ M, hydrolyses the peptide Leu-Trp-Met-Arg-Phe-Ala at the Arg-Phe bond and shows both chymotrypsin-like and trypsin-like specificity⁹. In contrast, our enzyme is not inhibited by

pCMB at 1 mM and does not cleave the peptides at the sites of single basic residues (for example, Tyr-Gly-Gly-Phe-Met-Arg-Phe-NH₂)³, indicating that our enzyme is not related to proteinase B. The fact that our enzyme was identified also in cell extracts prepared mechanically by Dyno-Mill treatment (not enzymatically with Zymolyase) excludes the possibility that our enzyme resulted from contamination of the Zymolyase preparation by the bacterial protease.

At present, nobody has a clear idea of how many different enzymes are involved in the processing, whether each hormone system has its own unique enzymes, or whether several generic enzymes accomplish most of the known cleavages. Therefore, identification of the enzyme candidates participating in the processing is important, and our pro-pheromone convertase Y should be compared directly with Thorner's enzyme.

K. MIZUNO
H. MATSUO

Department of Biochemistry,
Miyazaki Medical College,
Miyazaki, Kiyotake,
889-16 Japan

1. Docherty, K. & Steiner, D. F. *A. Rev. Physiol.* **44**, 625-638 (1982).
2. Loh, Y. P., Brownstein, M. J. & Gainer, H. A. *Rev. Neurosci.* **7**, 189-222 (1984).
3. Mizuno, K. & Matsuo, H. *Nature* **309**, 558-560 (1984).
4. Fletcher, D. J., Quigley, J. P., Bauer, G. E. & Noe, B. D. *J. Cell Biol.* **90**, 312-322 (1981).
5. Loh, Y. P. & Gainer, H. *Proc. natn. Acad. Sci. U.S.A.* **79**, 108-112 (1982).
6. Mizuno, K., Miyata, A., Kangawa, K. & Matsuo, H. *Biochem. biophys. Res. Commun.* **108**, 1235-1242 (1982).
7. Lindberg, I., Yang, H. Y. T. & Costa, E. *Biochem. biophys. Res. Commun.* **106**, 186-193 (1982).
8. Julius, D., Brake, A., Blair, L., Kunisawa, R. & Thorner, J. *J. Cell* **37**, 1075-1089 (1984).
9. Kominami, E., Hoffschulte, H., Leuschel, L., Maier, K. & Holzer, H. *Biochim. biophys. Acta* **661**, 136-141 (1981).

Water in the hydrocarbon core of micelles

ARE water molecules excluded from the hydrocarbon core of surfactant micelles, or can they penetrate in-between hydrocarbon chains? Dill *et al.*¹ give an answer based on the contrast variation method in neutron scattering. This method measures the zero-angle limit of the scattered intensity as a function of the scattering length density of the solvent. If their point of view is correct, it provides an important and general technique for studying the hydration and porosity of all colloidal particles. Unfortunately, their claim is based on a misinterpretation of the contrast variation method.

Indeed, for solutions of non-interacting particles, the zero-angle limit of the intensity is a purely thermodynamic quantity, related only to the mass of the dry particle, and contains no information on the arrangement of the constituents within the particle. Formally, it integrates the 'excess' scattering length density with respect to the solvent^{2,3}, $I(q \rightarrow 0) = AN(\Sigma b_i - \rho_s V)^2$, where A is an instru-

mental constant, N the number of particles, Σb_i the sum of the scattering lengths of all nuclei 'chosen' as belonging to the particle, V the corresponding volume of the particle and ρ_s the scattering length density of the solvent and q the scattering vector. If solvent molecules are included in the particle, they will contribute equally to Σb_i and to $\rho_s V$, unless their specific volume differs substantially from the bulk solvent, which would be extraordinary. Thus, their net effect on the intensity will be zero.

An equivalent formulation calculates the intensity from the 'average' scattering length density $\bar{\rho}$ of the particle; $I = AN(\bar{\rho}V - \rho_s V)^2$ with $\bar{\rho} = (\rho_p v_p + \rho_s v_s)/(v_p + v_s)$ and $V = v_p + v_s$, where v_p is the volume of the dry particle and v_s is that of the solvent included in it. Through simple algebra, this reduces to $I = AN[(\rho_p - \rho_s)v_p]^2$. Hence only the parameters v_p and ρ_p of the dry particle can be measured.

This formalism also applies to the contrast variation method, where the scattering density of the particle is matched by that of the solvent; the matching point will always be obtained for $\rho_s = \rho_p$. Hence, for micelles, it is the scattering density ρ_p of the dry micelle that is measured; solvent molecules in the micelle are not seen by this method.

We know only two ways of measuring solvent penetration in micelles through neutron scattering: one is to go to large scattering vectors ($0.6 \text{ \AA}^{-1}$), where the structure of the micelle can be resolved⁴; the other is to use concentrated solutions of strongly interacting micelles⁵, for then the hydration water increases the effective volume fraction, which becomes a thermodynamic quantity measured through $I(0)$. The general conclusion is that bound water is important (eight per headgroup) but is excluded from the core.

Thus, we believe that the author's conclusion is correct for reasons other than those given in their letter; their method does not, however, prove the point. This was also overlooked by others⁶, who have concluded that water is excluded from near the ionic headgroups of micelles. If this method was taken at face value for other colloidal systems it could lead to misleading results.

B. CABANE

Physique des Solides,
Université Paris-Sud,
91405 Orsay, France

T. ZEMB

Département de Physico-Chimie,
CEN-Saclay, 91191 Gif Sur Yvette,
Cedex, France

1. Dill, K. A. *et al. Nature* **309**, 42-45 (1984).
2. Jacrot, B. *Rep. Prog. Phys.* **39**, 911-953 (1976).
3. Sturmman, H. B. & Miller, A. J. *Appl. Crystallogr.* **11**, 325-345 (1978).
4. Cabane, B., Duplessix, R. & Zemb, T. in *Surfactants in Solution* (eds Mittal, K. & Lindman, B.) Vol. 1, 373-403 (Plenum, New York, 1984).
5. Hayter, J. B. & Zemb, T. *Chem. Phys. Lett.* **93**, 91-94 (1982).
6. Tabony, J. *Molec. Phys.* **51**, 975-989 (1984).

CHEN AND DILL REPLY—We agree with Cabane's point regarding the contrast variation technique, mentioned in the first paragraph on page 44 of our article¹. But we must emphasize that the original paper by Bendedouch *et al.*² did contain firm evidence that there is little penetration of water into the micellar core and that substantial hydration occurs at the outer layer of the micelle where the headgroups of the surfactant molecules reside.

Quoting the analysis procedure used in ref. 2, the differential scattering cross-section per unit volume, for a nearly mono-dispersed and nearly spherical system of micelles isotropically dispersed in solution, is

$$\frac{d\Sigma}{d\Omega}(q) = n_p P(q) S(q\sigma) \quad (1)$$

where n_p is the number of micelles per unit volume calculable from the surfactant concentration and the aggregation number, $P(q)$ the particle structure factor and $S(q\sigma)$ the interparticle structure factor. The information on micellar structure, aggregation number and hydration comes from both factors $P(q)$ and $S(q\sigma)$. $(d\Sigma/d\Omega)(q)$ is the differential cross-section per unit volume.

For experiments that measure the cross-section at 'zero' q and, for small q (such that $q \times \text{size} < 1$), one can write

$$P(q) = P(0) \exp(-q^2 R_g^2/3) \quad (2)$$

$$P(0) = \int_V d^3r (\rho(r) - \rho_s) \quad (3)$$

and

$$R_g^2 = (P(0))^{-1} \int_V d^3r r^2 (\rho(r) - \rho_s) \quad (4)$$

Using standard notation.

The contrast variation technique which is based on measurements of the zero angle intensity, $P(0)$, indeed contains no information on the solvent (water) penetration and the hydration, because in equation (3) the integration over the volume of the micelle (V) involves the difference of scattering length densities of the micelle and the solvent. Analysis of our 'internal contrast variation' data, more accurate than the 'external contrast variation' data, gives an aggregation number $\bar{n} = 78$ and a dry volume per monomer $V_m = 402 \text{ \AA}^3$ (refs 2, 3).

The analysis of the finite q data gives, from equation (2), the radius of gyration, R_g , which, by definition in equation (4), is related to the actual volume V of the micelle (as opposed to the dry volume) and hence would give information on the solvent penetration and hydration. Guinier plots of small q data² give the same value of $R_g = 15.4 \text{ \AA}$, independently of the degree of deuteration of the tail groups, which means that the scattering of neutrons is dominated by the hydrophobic tail part of LDS (lithium dodecyl sulphate) monomers forming the core of the micelle. If one assumes that the tails form a close-packed ellipsoidal core with

a semi-minor axis, b , equal to the fully stretched tail length, 16.7 Å, the semi-major axis, a , is then determined to be 23.4 Å using 350 Å³ as the steric volume of the hydrocarbon tail. The dry head group of the monomer would then occupy a volume equal to $V_H = 402 - 350 = 52$ Å³, which corresponds to a sphere of diameter $l = 4.6$ Å. Thus, the outer layer of the micelle can be assumed to be a shell of thickness l , consisting of 78 head groups plus solvent molecules. Such a model would predict that there are ~9 water molecules in the outer layer for every one of the head group. The radius of gyration calculated from this model is $R_g = 15.2 \pm 0.2$ Å, in agreement with the experimental value. If we now allow for one solvent molecule per monomer in the core, then, taking the volume of the water molecule to be 30 Å³, a similar calculation would give $R_g = 15.8$ Å, which disagrees with the experimental value².

Finally, systematic measurement of the cross-section in concentrated LDS solution⁴ allowed us to fit our model equation (1) consistently well. $S(qr)$, thus extracted, unambiguously gives $\sigma = 2(R + l)$, where R is calculated from the volume of a close-packed dry core and l can be anywhere in the range 4.6–5.6 Å, depending on slight variations of the micellar surface charges.

S. H. CHEN

Department of Nuclear Engineering,
Massachusetts Institute of Technology,
Cambridge,
Massachusetts 02139, USA

K. DILL

School of Pharmacy,
Department of Pharmaceutical Chemistry,
University of California,
San Francisco, California 94143, USA

1. Dill, K. A. *et al.* *Nature* **309**, 42–45 (1984).

2. Bendedouch, D., Chen, S. H. & Koehler, W. C. *J. phys. Chem.* **87**, 153–159 (1983).

3. Chen, S. H. & Lin, T. L. in *Methods of Experimental Physics—Neutron Scattering Vol. II* (eds Sköld, K. & Price, D. L.) Ch. 16 (Academic, New York, 1985).

4. Bendedouch, D., Chen, S. H. & Koehler, W. C. *J. phys. Chem.* **87**, 2621–2628 (1983).

Species variation in sensitivity of general anaesthesia to high pressure

BASED largely on the coincidence in crustacea of lack of pressure reversal of anaesthesia, insensitivity to strychnine and absence of glycine, and on similarities between strychnine- and high pressure-induced convulsions, Smith *et al.*¹ have proposed a hypothesis of general anaesthesia related to glycine receptors. Such an interpretation can be challenged on several grounds.

First, the authors' work¹ shows that the relative potencies of a series of general anaesthetics remain constant across a number of species, whether or not they have glycine receptors. Second, binding of strychnine at the glycine receptor in the

mammalian central nervous system is unaffected by general anaesthetics². Third, there is abundant neurophysiological data on the lack of interaction between anaesthetics and glycine. Short-latency, glycine-mediated synaptic inhibitions are not enhanced by general anaesthetics, whereas long-latency γ -aminobutyric acid (GABA)-mediated inhibitions are increased in both amplitude and duration³. In a variety of *in vivo* and *in vitro* experimental protocols, the inhibitory effects of glycine are not affected by anaesthetic doses that enhance GABA actions^{4–6}.

As most of the above experiments have concentrated on barbiturates, we have recently examined the effects of two other clinically useful anaesthetics on the mammalian CNS^{7,8}. We found no evidence to support the view that perturbation of the glycine system is a common property of general anaesthetics. The dissociative anaesthetic ketamine did not affect responses of neurones to glycine or GABA but did selectively block activation of the neurones by the glutamate analogue, *N*-methylaspartate⁷; correlated with this, ketamine had no clear effect on synaptic inhibitions but did reduce polysynaptic reflexes⁸. When tested on spinal reflexes and inhibitions in decerebrate cats⁸, the steroid anaesthetic, alphaxalone, clearly enhanced long-latency GABA-mediated inhibitions but had no effect on short-latency inhibitions, thought on other grounds to be mediated by glycine⁹.

It seems, therefore, that general anaesthetics do not influence the operation of glycinergic synapses. It remains possible that an action on the glycine system underlies the effects of high pressure on vertebrates. The absence of pressure-induced hyperactivity in crustacea¹, presumably an evolutionary advantage to subaquatic species, might support this view. In this case, the present models, which assume that pressure and anaesthetics act at the same site, would need to be re-appraised.

DAVID LODGE

Department of Physiology,
The Royal Veterinary College,
University of London,
Royal College Street,
London NW1 0TU, UK

1. Smith, E. B. *et al.* *Nature* **311**, 56–57 (1984).

2. Snyder, S. H. & Enna, S. J. *Adv. biochem. Psychopharmac.* **14**, 81–91 (1975).

3. Weakley, J. N. *et al.* *Archs int. Pharmacodyn.* **171**, 385 (1968).

4. Barker, J. L. & Ransom, B. R. *J. Physiol., Lond.* **280**, 355–372 (1978).

5. Evans, R. H. & Hill, R. G. *Experientia* **34**, 1325 (1978).

6. Lodge, D. & Curtis, D. R. *Neurosci. Lett.* **8**, 125–129 (1978).

7. Anis, N. A. *et al.* *Br. J. Pharmac.* **79**, 565–575 (1983).

8. Lodge, D. & Anis, N. A. *Br. J. Anaesth.* **56**, 1143–1151 (1984).

9. Curtis, D. R. & Johnston, G. A. R. *Ergeon. Physiol.* **69**, 97–184 (1974).

SMITH *ET AL.* REPLY—The eloquent points raised by Lodge are both valid and stimulating. As stated in our paper¹, the finding that the pressure reversal of

anaesthesia appears to be linked to strychnine sensitivity can be interpreted in more than one way.

If the extended unitary hypothesis (which presupposes that pressure and all general anaesthetics share a common mechanism) is accepted², our observations point to strychnine-sensitive processes as an important element in the 'loss of consciousness' in vertebrates. The most widely accepted view would then implicate glycinergic processes. Implicit in this view is that the loss of swimming activity in shrimps, at comparable anaesthetic doses, must arise via a different mechanism. Indeed, our additional finding that shrimps appear to have a dose-response curve that is less steep than that observed for anaesthesia in vertebrates would be consistent with this view.

However, we cannot exclude the possibility that the pressure reversal of anaesthesia could arise from an independent action of pressure resulting in a physiological rather than a pharmacological antagonism of anaesthesia; this is compatible with the view of Lodge, who quite rightly points out that there is no direct evidence that general anaesthetics potentiate the action of glycine-mediated inhibition, although it has been demonstrated that the anaesthetics ketamine and althesin confer substantial protection against strychnine³. However, if Lodge's work, at the level of the spinal cord, is considered relevant to loss of consciousness which results from the action of anaesthetics at higher levels within the neuro-axis, then the striking diversity of responses to different agents must lead to a rejection of the unitary hypothesis. This implies that different anaesthetics can act by different mechanisms. Any rejection of such a powerful generalization—the unitary hypothesis—that has provided the key to interpreting the common effects of a disparate group of anaesthetic substances, will require an unequivocal body of evidence.

Whichever interpretation of our results is correct, both are incompatible with present thinking—that general anaesthetics act by inducing a general perturbation of cellular membranes—and we believe that models involving more specific molecular interactions are more appropriate.

E. B. SMITH
F. BOWSER-RILEY
S. DANIELS
I. T. DUNBAR
C. B. HARRISON
W. D. M. PATON

Physical Chemistry Laboratory,
University of Oxford,
South Parks Road,
Oxford OX1 3QZ, UK

1. Smith, E. B. *et al.* *Nature* **311**, 56–57 (1984).

2. Miller, J. C. & Miller, K. W. in *Physiological and Pharmacological Biochemistry* Vol. 12 (ed. Blaschko, H. K. F.) 33–75 (Butterworths, London, 1975).

3. Wardley-Smith, B., Little, H. J. & Halsey, M. J. *Br. J. Anaesth.* **55**, 235–236 (1983).

Fundamental developments

R.H. Dalitz

Constructing Quarks: A Sociological History of Particle Physics.

By Andrew Pickering.

Edinburgh University Press/University of Chicago Press: 1984. Pp.468. £20, \$30.

THE first curious thing about this book is its title. The jacket makes its meaning clear: "scientists are not passive observers . . . of nature, but active constructors of the world of natural phenomena . . .". The second curious thing is that there is nothing inside the book which justifies this conclusion.

The author has a background in theoretical particle physics but is now working as a sociologist of science. His account of the history of experiment and theory in particle physics is both succinct and accurate, and while the book is not written in a popular style it is also not technical; any interested science undergraduate could read it with considerable understanding. One of its threads is to relate the approach and contribution of each of the theoreticians centrally involved to his educational background and previous research experience. These short biographies are factual and interesting; the reader is left to draw his own conclusions. The main theme, however, is that implied by the book's title, as I shall discuss below.

Through the past decade there has been a shift of emphasis in fundamental physics, from hadrons (mesons and baryons) to quarks, largely triggered by the startling discovery of the J/ψ particle in November 1974, although already implicit in our knowledge of hadron spectroscopy (discussed here in Chapter 4) and of deep inelastic scattering of leptons by protons and neutrons (presented in Chapter 5) more than five years earlier. Early in 1974, theoreticians had already predicted from the empirical absence of neutral weak currents involving strangeness change that there should exist a fourth quark c (c = "charm", the first three quarks being u = "up", d = "down" and s = "strange"), estimating its mass to be about 1.5 GeV. The empirical mass for J/ψ was 3.1 GeV and J/ψ soon became understood as a bound state of c with its antiparticle $\bar{c}$, and so provided us with our first "hydrogen atom of quark physics". Particles with non-zero charm, for example mesons with quark structure ($\bar{c}u$), ($\bar{c}d$) or ($\bar{c}s$), were empirically elusive, but the theoreticians insisted on them. The experimenters then worked hard to find them, and their search reached success in 1976, after they adopted a more sophisticated analysis procedure. Chapter 9 gives a good account of these charm discoveries. A fifth quark (b = "bottom") was established in 1977, through the discovery of the T (upsilon) particle, and current experiments suggest the existence of a sixth quark (perhaps t =

"top"). The masses of these quarks range from several MeV for u and d , to 4.5 GeV for b and about 40 GeV for t .

A deep, elegant and essentially unique theory of the strong interactions of quarks has been developed as a gauge theory based on a symmetry already indicated by the data when we knew only the quarks (u, d, s), a symmetry involving a new degree of freedom for quarks, expressible in terms of a three-dimensional space whose axes are whimsically specified by colours. This theory, discussed in some detail in Chapter 7, is now known as quantum chromodynamics (QCD) and the symmetry as $SU(3)_C$. The gauge field is vector and its quanta are termed gluons. QCD appears to imply that quarks and gluons can only exist within bound states which have no net colour, a constraint known as quark confinement. Since quarks have fractional charge values, they would be readily apparent in the free state, whereas no such objects have yet been established.

Another fundamental step forward was the unification of electromagnetic and weak interactions of quarks and leptons as the electroweak theory, which takes the form of a spontaneously-broken gauge theory based on an $SU(2) \times U(1)$ symmetry. This theory was first laid out in 1967 and was proved acceptably finite in 1971. Now known as the Standard Model, in its simplest form it required the existence of some very massive weak bosons ($W^\pm$ and Z^0), conceptually partners to the photon, which couple with electromagnetic strength to quark pairs as well as to lepton pairs. This theory also predicted neutral weak currents with no strangeness change; at the prompting of theoreticians (but after a period of uncertainty), the experimenters established the existence of these currents in 1974. The history of gauge theories and electroweak unification is given here at length in Chapter 6; the Standard Model is covered in Chapter 10. The $W^\pm$ and Z^0 bosons were identified and measured in 1983, just as this book was going to press, and show excellent accord with the Standard Model.

Although quarks and gluons are confined, quark-quark collisions with high energy- and momentum-transfer occur in very high energy collisions between hadrons. Each secondary quark generates a jet of hadrons which carry its momentum, and the characteristics of quark-quark scattering can be deduced from statistics on these jets. For example, quark-quark scattering is now known to

follow Rutherford's Law for spin- $\frac{1}{2}$ fermions. Again, weak bosons can decay to a quark-antiquark pair, each generating a quark jet, so leading to phenomena involving correlated jets. In a hadron containing a heavy quark, this quark may transform to a lighter quark with the emission of a pair of leptons, the secondary quark generating a jet of hadrons. There are many possible situations for study in this new area of heavy quark processes.

After reviewing at length the historical development of particle physics, as well as the present scene, the author becomes much exercised by the "incommensurability" of "old physics" and "new physics". The former, typical of the pre-1974 era, is better termed "hadron physics", being the study of the interactions of mesons and baryons, now recognized as "colour-less" composite particles made up of quarks and antiquarks interacting through the gluon field. The latter is better named "quark-lepton physics", where experimental observations are interpreted directly in terms of the fundamental quark-gluon, quark-boson and boson-lepton interactions. This transition to quark-lepton physics has been possible largely because these basic entities appear point-like, so that hard collisions occur at a measurable rate. A similar change of emphasis took place around 1950, when the forefront of fundamental physics moved from nuclear phenomena (the interactions between systems of nucleons) to hadron phenomena (starting with meson-proton scattering), and the accelerators for its study had to be built on the energy scale 1,000 MeV rather than 10 MeV. The simpler phenomena in the physics of hadrons — their detailed spectroscopy, their electromagnetic properties and their interactions at moderate energy — have now been accounted for in considerable detail on the basis of QCD and it is generally anticipated that all of hadron physics, up to moderate energies (say, 10 to 20 GeV), will ultimately be understood in this way, if sufficient theoretical calculation is carried out. For the present, however, we must get on with the study of quark-lepton physics, where the phenomena are much simpler and can teach us more directly about the fundamental interactions.

The author is puzzled at the lack of controversy among physicists about this transition from the study of hadron physics to the study of quark-gluon physics. In the final chapter, entitled "Producing a World", he states: "The history of HEP [high energy physics] can be understood as a communal search for a congenial world: a world which made social sense and in which practice could be socially organised". He concludes that this transition has been a matter of tacit choice, that particle physics today is essentially the result of a conspiracy between experimenters and theoreticians. The theoreticians demand certain phenomena, for their own reasons,

and the experimenters then build equipment sensitive only to those phenomena. As Pickering puts it: "detectors are [now] designed to optimize new physics and to exclude old physics. So specialized have they become that their sole use lies in the investigation of rare phenomena". The experimenters establish what has been required of them, and the theoreticians move on to further demands, in "a social symbiosis of experimental and theoretical practice".

Of course, it must be said here that the theoreticians have had a very good run since about 1970. The gauge principle has proved extraordinarily successful in providing theories which are not *ad hoc* but rooted in deep symmetry principles. But while it is also true that the experimenters have made mistakes, and have had to learn how to deal with background effects which can fog their data on the phenomena they seek to explore, they are not subservient to the theoreticians. In reality, experimenters are cussed individuals, eager to prove the theoreticians wrong whenever possible. In any event, what the author has overlooked is that the experimenters will not be able to find what the theoreticians desire of them, when the theory concerned does not correspond to the real world. Experimenters have the capability of disproving theories, that is the strength of their position, and of course most theories are wrong.

The very direct relationship of the phenomena now seen in the regime of large energy- and momentum-transfer with the underlying theoretical entities and the interactions between them, suggests that particle physics may now have reached a regime of simplicity, as so often hoped for in the past although in a different form. There is no compelling reason why any asymptotic regime of simplicity should exist, of course. This regime stands out only because of the point-like character of the quarks and leptons, relative to the size of about 10^{-13} cm typical for hadrons. As things are, events in this regime are quite rare and it would not have been possible to find them at all amidst the background of events arising from the processes of hadronic physics, if the quark and lepton interactions were suppressed by form factors associated with finite size.

There is, of course, a problem in the minds of many concerning the confinement of quarks and gluons. However, the interpretation of phenomena in terms of entities which we cannot exhibit at the time is not a new situation in physics. The atomic theory of gases was used fruitfully for some decades before a single atom was identified. What is new is that the fundamental entities within hadrons are quarks which we cannot hope *ever* to exhibit individually. Are quarks real, in this situation? This is a nice point for philosophical discussion, but in the laboratory there is no doubt at all. Quarks allow us to interpret in simple terms what we see in experiments in this regime. Calculations based on them

and their interactions fit the data. They allow us to design new experiments and to decide which measurements are likely to be the most informative. After quite a short period, the active physicist treats them, and thinks of them, as real objects. They have an operational reality for him. On the other hand, these quarks, leptons and bosons are still not necessarily fundamental particles, and the future course of quark-lepton physics is uncertain. Will history run through another cycle and lead us to find that these particles are themselves composite? We see no clear indication for compositeness at present, although there are recent reports of rare anomalous processes in quark-lepton physics for which we have no explanation to hand.

The strange thing about this book is that it is mostly rather good, well-written and engaging, until the author goes off the rails with the idea that the experimenters and theoreticians are making up a world to suit themselves. He is obviously upset that the kind of physics that he learned, "hadron physics", is being ignored in today's experimentation, which does indeed endeavour to eliminate as much of its effects as possible in order to get down to the underlying fundamental physics. Pickering does not seem to have understood the transition which he has been observing, nor to realize that this has happened time and again in the course of physics. However, the sceptical reader will find the book quite rewarding, provided he skips the first and last chapters or takes due warning to read them with caution. □

R.H. Dalitz is Royal Society Research Professor in the Department of Theoretical Physics, University of Oxford.

Channels defined

Charles F. Stevens

Ionic Channels of Excitable Membranes.

By Bertil Hille.

Sinauer/Blackwell Scientific: 1984. Pp. 426. \$32.50, £29.50.

NEUROBIOLOGY has made great strides in the past decade or two, and progress in understanding neuronal electrical activity has been particularly impressive. Bertil Hille's new book provides us with an elegant and lucid account of these recent advances in knowledge about electrical excitability.

What is a channel? When Hodgkin and Huxley showed how the nerve impulse arises from increases in the neuronal membrane's permeability to sodium and to potassium ions, these ions were generally viewed as passing through tiny membrane pores that open in response to changes in the transmembrane voltage. In recognition of the hypothetical nature of these rather

mysterious "pores", however, the term "channel" soon came into use; "pore" connotes physical characteristics that the vaguer term "channel" does not and, in the early days when we had no way of directly studying the microscopic processes of ion permeation, the less committal name seemed safer. Over the past ten years, we have discovered that channels are integral membrane proteins in which a microscopic pore opens and closes to regulate the flow of ions across the membrane.

In the old days, two questions about channels were clear. How is gating achieved? And how can a channel select between different ionic species? That is, by what mechanism does a channel open and close its pore, and how can that pore — in a potassium selective channel, for example — let potassium ions (crystal radius = 0.133 nm) pass through while excluding sodium ions (crystal radius = 0.095 nm)? Hille's book gives the modern answers to these classical questions and also deals with more recent issues. What is the structure of channel proteins? How are they synthesized and inserted in the right membranes? How did channels evolve? In what ways are various channel types different and what properties do they share? More specialized sections survey our understanding of chemical and toxin modification of channel gating and of the way in which small molecules of the right shape and size can block pores. Introductory sections cover the required background of physical and chemical principles, and also the classical descriptions of channel behaviour such as the Hodgkin-Huxley model.

The book is written in a clear and crisp style with excellent illustrations and a lot of references (about a thousand of them), ranging from work done in the nineteenth century to articles published in the past five years (about a third of the citations). Chapters are relatively independent, are designed for skipping around in and between, and are arranged in short sections with headlines ("Channels are Needed for Excitation"); "'Hydrated Radius' is a Fuzzy Concept"; "Channel Evolution is Slow").

Not only does Hille's book present information clearly, but it fills an important void: no other systematic and modern treatment of this material is available. And while it is not a Sunday-afternoon-learn-a-bit-about-the-brain book, it does make the modern view of channels available to all neuroscientists — advanced undergraduates, graduate students and scientists who need to know what we understand about channels. Hille has been a leading contributor to research on channels, and has brought the expert's insight, together with the master teacher's skill, to the job of explaining what is known about the basis of neuronal electrical activity.

Charles F. Stevens is a Professor of Physiology at Yale University School of Medicine.

The changing face of the Earth

Robert P. Beckinsale

A Short History of Geomorphology.

By Keith J. Tinkler.

Croom Helm/Littlefield, Adams: 1985. Pp.317. £19.95, \$25.

THE compilation of a short, comprehensive and readable history of the study of landforms calls for selective wisdom of the highest class. Keith Tinkler's book has all those virtues, even though nearly one-quarter of it is given over to an excellent bibliography and a rather simple index. The dominant method used is to illustrate particular themes by studying influential publications in some depth to convey an overall view of the subject without sacrificing attention to detail. Necessarily, where possible, the focus falls upon form and process as viewed over time.

The reader is assumed to have at least a nodding acquaintance with geomorphology and its terminology, but these prerequisite demands are small and undergraduates are encouraged also by a brief historical *caveat*. The account moves quickly through classical authors to the Italian Renaissance and so to the long period when the earth sciences, with rare exceptions, were constrained by biblical notions. This period merged into the age of James Hutton and John Playfair who believed that "in nature we have no deficiency of time" and whose thoughts were as complementary as their styles were disparate.

There followed the effect of the Industrial Revolution on scientific thinking, which fostered Charles Lyell, the great uniformitarian, who used existing phenomena as a complete basis for his conclusions on

processes (and not surprisingly sometimes showed an unfortunate bias). Before his death in 1875, fluvialism had become more positive and the reality of a recent Ice Age generally accepted, thanks to "a blend of factual investigation and imaginative insight... unparalleled in the history of geomorphology up to that point" (p.119).

Most of the remainder of the text covers geomorphology since the 1880s rightly emphasizing the influence of Eduard Suess who postulated a periodic movement of sea-level, diastrophically controlled and globally synchronous, and of William Morris Davis who popularized stages in river courses and cycles in the development of a variety of landforms. During and after the Second World War allegiance to marine planation and cyclic aspects faded in face of improved technologies and heightened interest in climato-genetic geomorphology and in details of fluvial, coastal and tropical landforms. Since 1960 the shift towards quantification and process studies has quickened and progress has been made in, for example, the understanding of slope development, river flow, ice-sheet dynamics and the significance of physical thresholds. Conceptual and theoretical advances have included the systems theory with its concern with a stable, though not static, equilibrium state.

In the final chapter Tinkler tries to assess future trends, a task which some readers may consider regrettable as this space could have been more profitably devoted to ocean-floor spreading, plate subduction, vulcanism and orogenesis, topics which are almost ignored. But anyone interested in the study of landforms will find this a competent summary which manages to sustain a stimulating commentary throughout its commendably short course. □

Robert P. Beckinsale was formerly a Lecturer in Geography at the University of Oxford.

A chip off the old block

E.N.K. Clarkson

Living Fossils.

Edited by Niles Eldredge and Steven M. Stanley.

Springer-Verlag: 1984. Pp.291. DM138, \$54.10.

IN SOME groups of animals and plants the rate of evolutionary change is exceedingly slow, and their modern representatives hardly differ structurally from the earliest members to appear in the fossil record. G.G. Simpson referred to such slow evolution and abnormally long survivorship as bradytely, distinguishing it qualitatively from horotelic (normal) or tachytely (very rapid) evolutionary change. The survivors of such bradytely lines are "living fossils",

conceived as either specifically adapted to particular niches within a more or less permanent environment or as more than usually tolerant of fluctuating conditions. Lineages which become bradytely might be "advanced" on their first appearance but lack of environmental stimulus for change would enable them to survive indefinitely.

How valid are Simpson's concepts in the light of the current developments in patterns and processes of macroevolution? Is there anything special about such arrested evolution or is bradytely simply the "slow end" of a normal distribution of evolutionary events?

Eldredge and Stanley, in trying to come to terms with these and related questions, solicited specialist contributions to obtain data on groups which might be, or have been, regarded as living fossils. They requested, for example, information on anatomical similarity of modern forms to their ancient relatives, the time scale involved, ecological specializations, diversity and so

on. In other words, how good are the examples of living fossils and in evolutionary terms what does it all mean?

There are 30 separate papers in this collection and two concluding summaries, one by each editor. Over half of the papers are devoted to vertebrates — various mammals, coelacanths, *Amia* and *Polypterus* (though not *Sphenodon*). The interesting question is asked (and answered) as to whether there are any anthropoid living fossils. A great variety of invertebrates are discussed, among them *Neotrigonia*, *Peripatus* and limulines (though not *Lingula*), but also bryozoans, and cephalocarids as living fossils lacking a fossil record. There are, however, no plants, not even *Ginkgo*.

Some authors have given a largely anatomical account of their group, but have usually tried to assess whether the surviving representatives are or are not "good" living fossils. Others have also advanced concepts and theory, often thought provoking. It is, of course, easier to categorize as a living fossil an animal whose similar forebears appeared 500 million years ago, than a type which originated more recently. Even then it may not be easy, for, as Ward, for example, acknowledges in his excellent paper on *Nautilus*, the apparent stasis may only be a factor of low morphological complexity. And again Fisher points out that the sparse fossil record of limulines virtually precludes their evaluation as "good" living fossils. In spite of the very different ways in which the various authors have approached their groups, this volume is a really valuable source of well researched information.

Having collected the evidence, to what conclusions do the editors come as to the reality of bradytely as categorically distinct from other rate-phenomena? Eldredge concludes that there is no strong evidence for bradytely as a separate kind of evolutionary mode. Likewise Stanley suggests that Simpson's three classes, including bradytely (originally developed within a gradualistic framework) tend to lose their value as separate categories when seen from a punctationist standpoint. Does this imply that, after all, one's own point of view may still be the ultimate determinant, and the question should be left open? Possibly, but few could take exception to the concept that living fossils are simply survivors of long-lived groups which have continued, for a variety of reasons, including those Simpson proposed. □

E.N.K. Clarkson is Reader in Geology at the University of Edinburgh.

New in paperback

● *Ghosts in the Mind's Machine: Creating and Using Images in the Brain* by Stephen Michael Kosslyn. Publisher is W.W. Norton, price is £9.95. For review see *Nature* 310, 344 (1984).

● *The Youngest Science* by Lewis Thomas. Publisher is Oxford University Press, price is £3.95. For review see *Nature* 302, 778 (1983).

Mathematics

Algebra Through Practice, 3 Vols. Book 1 Sets, Relations and Mappings. Book 2 Matrices and Vector Spaces. Book 3 Groups, Rings and Fields. By T.S. BLYTH and E.F. ROBERTSON. Cambridge University Press: 1984. Book 1: pp.97, pbk ISBN 0-521-27285-8; Book 2: pp. 99, pbk ISBN 0-521-27286-6; Book 3: pp. 95, pbk ISBN 0-521-27288-2. Pbk £3.50 each.

Developments in Statistics, Vol. 4. PARUCHURI R. KRISHNAIAH (ed.). Academic: 1983. Pp. 287. ISBN 0-12-426604-5. \$62.

Fundamentals of Mathematics, Vols 1 & 2. H. BEHNKE *et al.* (eds). MIT Press: 1984. Vol. 1: pp. 549, ISBN 0-262-52093-1; Vol. 2: pp. 685, ISBN 0-262-52094-X. £14.25 each.

Fundamentals of Mathematics, Vol. 3 Analysis. H. BEHNKE *et al.* (eds). MIT: 1983. Pp. 541. Pbk ISBN 0-262-52095-8. Np.

Mathematical Magic Show. By MARTIN GARDNER. Viking: 1984. Pp. 284. ISBN 0-670-80327-8. £9.95.

Methods of Numerical Integration, 2nd Edn. By PHILIP J. DAVIS and PHILIP RABINOWITZ. Academic: 1984. Pp. 612. ISBN 0-12-206360-0. \$52, £36.50.

Multilinear Algebra. By D.G. NORTHCOTT. Cambridge University Press: 1984. Pp. 198. ISBN 0-521-26269-0. £20, \$39.50.

Multivariate Analysis: Methods and Applications. By WILLIAM R. DILLON and MATTHEW GOLDSTEIN. Wiley: 1984. Pp. 587. ISBN 0-471-08317-8. £38.85.

Statistical Inference Based on Ranks. By THOMAS P. HETTMANSPERGER. Wiley: 1984. Pp. 323. ISBN 0-471-88474-X. £38.25.

Theory of Correspondences: Including Applications to Mathematical Economics. By ERWIN KLEIN and ANTHONY C. THOMPSON. Wiley: 1984. Pp. 256. ISBN 0-471-88016-7. £40.85.

Space Science

The Light-Hearted Astronomer. By KEN FULTON. Astro Media: 1984. Pp. 115. ISBN 0-913135-01-1. \$6.95.

Physics of Planetary Interiors. By G.H.A. COLE. Adam Hilger: 1984. Pp. 208. Hbk ISBN 0-85274-444-4; pbk ISBN 0-85274-445. Hbk £22; pbk £9.95.

The Universe of Galaxies: Readings from Scientific American. PAUL W. HODGE (ed.). W.H. Freeman: 1984. Pp. 113. Hbk ISBN 0-7167-1675-5; pbk ISBN 0-7167-1676-3. Hbk \$20.95; pbk \$10.95.

Physics

The Atomic Nucleus. By J.M. REID. Manchester University Press: 1984. Pp.279. Pbk ISBN 0-7190-0978-2. Pbk £6.50.

Bosons in Nuclei. D.H. FENG, S. PITTEL and M. VALLIERES (eds). Wiley: 1984. Pp. 238. Hbk ISBN 0-9971-950-18-9; pbk ISBN 0-9971-950-19-7. £33.90.

Classical General Relativity. W.B. BONNOR, J.N. ISLAM and M.A.H. MACCALLUM (eds). Cambridge University Press: 1984. Pp. 269. ISBN 0-521-26747-1. £25, \$44.50.

Coaxial A.C. Bridges. By B.P. KIBBLE and G.H. RAYNER. Adam Hilger: 1984. Pp. 203. ISBN 0-85274-389-0. £25.

Coupled Mode Theory: As Applied to Microwave and Optical Transmission. By HUANG HUNG-CHIA. VNU Science Press: 1984. Pp. 364. ISBN 90-6764-033-6. DM 118.

Group Theory in Physics, Vol. 1. By J.F. CORNWELL. Academic: 1984. Pp. 371. ISBN 0-12-189801-6. \$75, £49.50.

Electrochemical Detectors: Fundamental Aspects and Analytical Applications. T.H. RYAN (ed.). Plenum: 1984. Pp. 172. ISBN 0-306-41727-8. \$39.50.

Electron-Molecule Interactions and their Applications, Vol. 2. L.G. CHRIDTOPHOROU (ed.). Academic: 1984. Pp. 678. ISBN 0-12-174402-7. \$85, £59.50.

The Excitation and Propagation of Elastic Waves. By J.A. HUDSON. Cambridge University Press: 1984. Pp. 224. Pbk ISBN 0-521-31867-X. Pbk £9.95, \$19.95.

From Falling Bodies to Radio Waves: Classical Physicists and Their Discoveries. By EMILIO SEGRE. W.H. Freeman: 1984. Pp. 298. Hbk ISBN 0-7167-1481-7; pbk ISBN 0-7167-1482-5. Hbk £24.95; pbk £13.95.

Frontiers in Particle Physics. D. SIJACKI *et al.* (eds). Wiley: 1984. Pp. 416. ISBN 9971-950-57-X. £52.90.

Optical Radiation Detectors. By EUSTACE L. DERENIAK and DEVON G. CROWE. Wiley: 1984. Pp.300. ISBN 0-471-89797-3. £49.

Optics and Lasers. By MATT YOUNG. Springer-Verlag: 1984. Pp.269. ISBN 3-540-13014-4. DM 64, \$22.50.

Phase Transitions and Critical Phenomena. C. DOMB and J.L. LEBOWITZ (eds). Academic: 1984. Pp.318. ISBN 0-12-220309-7. \$60, £38.50.

The Physics of Submicron Structures. H.L. GRUBIN *et al.* (eds). Plenum: 1984. Pp.360. ISBN 0-306-41715-4. Np.

Power Semiconductors. By MILAN KUBAT. Springer-Verlag: 1984. Pp.507. ISBN 3-540-12569-8/0-387-12569-8. DM 99, \$38.50.

Pulsed Light Sources. By I.S. MARSHAK. Consultants Bureau: 1984. Pp.461. ISBN 0-306-10976-X. Np.

Relativity and Engineering. By J. VAN BLADEL. Springer-Verlag: 1984. Pp.402. ISBN 3-540-12561-2/0-387-12561-2. DM 80, \$28.10.

Solutions of Einstein's Equations: Techniques and Results. C. HOENSELLAERS and W. DIETZ (eds). Springer-Verlag: 1984. Pp.439. Pbk ISBN 3-540-13366-6/0-387-13366-6. DM 59, \$20.70.

Superconductivity in Magnetic and Exotic Materials. T. MATSUBARA and A. KOTANI (eds). Springer-Verlag: 1984. Pp.211. ISBN 3-540-13324-0. DM 65, \$23.70.

Symmetries in Particle Physics. ITZHAK BARS, ALAN CHODOS and CHIA-HSIUNG TZE (eds). Plenum: 1984. Pp.309. ISBN 0-306-41801-0. \$75.

Theory of NMR Parameters. By I. ANDO and G.A. WEBB. Academic: 1983. Pp.217. ISBN 0-12-056820-9. \$52, £30.

Treatise on Heavy-Ion Science, Vol.1: Elastic and Quasi-Elastic Phenomena. D. ALLAN BROMLEY (ed.). Plenum: 1984. Pp.753. ISBN 0-306-41571-2. \$95.

Vibronic Coupling: The Interaction between the Electronic and Nuclear Motions. By GAD FISCHER. Academic: 1984. Pp.222. ISBN 0-12-257240-8. £28.50, \$42.

Chemistry

Activation of Saturated Hydrocarbons by Transition Metal Complexes. By A.E. SHILOV. Reidel: 1984. Pp.203. ISBN 90-277-1628-5. Np.

Azides and Nitrenes: Reactivity and Utility. ERIC F.V. SCRIVEN (ed.). Academic: 1984. Pp.542. ISBN 0-12-633480-3. \$99.50, £70.

The Chemistry of Optically Active Sulfur Compounds. By ABRAHAM NUDELMAN. Gordon and Breach: 1984. Pp.253. Pbk ISBN 0-677-16390-8. Pbk \$58.

Comprehensive Treatise of Electrochemistry. RALPH E. WHITE *et al.* (eds). Plenum: 1984. Pp.620. ISBN 0-306-41448-1. \$89.50.

Comprehensive Treatise of Electrochemistry. Electrodes: Experimental Techniques. ERNEST YEAGER *et al.* (eds). Plenum: 1984. Pp.451. ISBN 0-306-41570-4. Np.

Contemporary Topics in Polymer Science, Vol. 5. E.J. VANDENBERG (ed.). Plenum: 1984. Pp.458. ISBN 0-306-41665-4. \$75.

Dynamics of First-Order Phase Transitions in Equilibrium and Nonequilibrium Systems. By S.W. KOCH. Springer-Verlag: 1984. Pp.148. Pbk ISBN 3-540-13379-8/0-387-13379-8. DM 19, \$7.

Growth of Crystals, Vol. 12. A.A. CHERNOV (ed.). Consultants Bureau: 1984. Pp.355. ISBN 0-306-18112-6. \$55.

Kinetics of Heterogeneous Catalytic Reactions. By MICHAEL BOUDART and G. DJEGMARIADASSOU. Princeton University Press: 1984. Pp.222. ISBN 0-691-08346-0. £32.40.

Methods and Applications in Crystallographic Computing. S.R. HALL and T. ASIDA (eds). Clarendon Press: 1984. Pp.506. ISBN 0-19-855190-8. £25.

Polyimides: Synthesis, Characterization and Applications. K.L. MITTAL (ed.). Plenum: 1984. Pp.614. ISBN 0-306-41670-0. Np.

Polymer Additives. JIRIE KRESTA (ed.). Plenum: 1984. Pp.408. ISBN 0-306-41807-X. \$72.50.

Polymer Blends: Processing, Morphology and Properties, Vol. 2. M. KRYSZEWSKI, A. GALESKI and E. MARTYSCCELLI (eds). Plenum: 1984. Pp.287. ISBN 0-306-41802-9. \$52.50.

Proton and Carbon NMR Spectra of Polymers, Vol. 3. By QUANG-THO PHAM *et al.* Wiley: 1984. Pp.677. ISBN 0-471-90394-9. £75.

Reductions in Organic Chemistry. By MILOŠ HUDLICKÝ. Ellis Horwood: 1984. Pp.309. ISBN 0-85312-345-4. £35.

Biological Sciences

Evolutionary Biology, Vol.18. MAX K. HECHT *et al.* (eds). Plenum: 1984. Pp.267. ISBN 0-306-41760-X. \$37.50.

A Field Guide to the Moths of Eastern North America. By CHARLES V. COVELL. Houghton Mifflin: 1984. Pp.496. Hbk ISBN 0-395-26056-6; pbk ISBN 0-395-36100-1. Hbk \$18.95; pbk \$13.95.

Fish Reproduction: Strategies and Tactics. G.W. POTTS and R.J. WOOTTON (eds). Academic: 1984. Pp.410. ISBN 0-12-563660-1. \$49, £29.50.

Flooding and Plant Growth. T.T. KOZLOWSKI (ed.). Academic: 1984. Pp.356. ISBN 0-12-424120-4. \$55, £42.50.

Foreign Compound Metabolism. J. CALDWELL and G.D. PAULSON (eds). Taylor & Francis: 1984. Pp.328. ISBN 0-85066-271-0. £32.

Forest Trees of Australia. By D.J. BOLAND *et al.* Nelson CSIRO: 1984. Pp.687. ISBN 0-17-006264-3. \$39.95.

Foundations of Sensory Science. W.W. DAWSON and J.M. ENOCH (eds). Springer-Verlag: 1984. Pp.577. ISBN 3-540-12967-7/0-387-12967-7. DM 288, \$104.90.

The Fragmented Forest: Island Biogeography Theory and the Preservation of Biotic Diversity. BY LARRY D. HARRIS. University of Chicago Press: 1984. Pp.211. Pbk ISBN 0-226-31764-1. Pbk £11.

Freshwater Biological Monitoring. D. PASCOE and R.W. EDWARDS (eds). Pergamon: 1984. Pp.167. ISBN 0-08-03231308. £22.50, \$35.

A Functional Biology of Sticklebacks. By R.J. WOOTTON. Croom Helm: 1984. Pp.265. ISBN 0-7099-2785-1. £17.95.

Fundamentals of Plant Pathology, 2nd Edn. By D. A. ROBERTS and P. W. BOOTHROYD. W.H. Freeman: 1984. Pp.432. ISBN 0-7167-1505-8. £29.95.

General

Agreement and Anaphora: A Study of the Role of Pronouns in Syntax and Discourse. By PETER BOSCH. Academic: 1983. Pp.260. ISBN 0-12-118820-5. Np.

Black Apollo of Science: The Life of Ernest Everett Just. By KENNETH R. MANNING. Oxford University Press: 1984. Pp.397. ISBN 0-19-503299-3. £27.

Scientific Research and Social Goals: Towards a New Development Model. FEDERICO MAYOR (ed.). Pergamon: 1982. Pp. 236. ISBN 0-08-028118-4. Np.

Syntax and Semantics, Vol. 8. PETER COLE and JERROLD M. SADOCK (eds). Academic: 1984. Pp. 347. ISBN 0-12-613508-8. \$65.00, £50.50.

Tahoe: An Environmental History. By DOUGLAS H. STRONG. University of Nebraska Press: 1984. Pp. 252. ISBN 0-8032-4141-0. \$16.95.

Technological Innovation: University Roles. The Association of Commonwealth Universities, John Foster House, 36 Gordon Square, London WC1H 0PF: 1984. Pp. 431. Pbk ISBN 0-85143-087-2. Pbk £6.

Total Mean Curvature and Submanifolds of Finite Type. By BANG-YEN CHEN. World Scientific: 1984. Pp. 352. Hbk ISBN 9971-966-02-6; pbk ISBN 9971-966-03-4. Hbk np; pbk £15.70.